IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Serial No. : 10/054,300

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Filed : January 22, 2002

For : NOVEL BICYCLONUCLEOSIDE ANALOGUES

Art Unit : 1623

Examiner : Travis C. McIntosh III

Docket No. : 01834CIP/HG

Customer No.: 01933

Confirm No. : 5360

STATEMENT OF ACCURACY OF TRANSLATION
(37 C.F.R. 1.55 & 1.68)

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MAIL STOP AMENDMENT

S I R :

The undersigned translator, having an office at

states that:

(1) I am fully conversant both with the Japanese and English languages.

(2) I have carefully compared the attached English language translation of International Patent Application Number PCT/JP00/04902, filed July 21, 2002 with the original Japanese

language patent application.

(3) The translation is, to the best of my knowledge and belief, an accurate translation from the original into the English language.

Date: August 18, 2006 Takamitsu Yoneda

Takamitsu YONEDA

(Type name of translator above)

Description

NOVEL BICYCLONUCLEOSIDE ANALOGUES

TECHNICAL FIELD

This invention relates to novel bicyclonucleoside analogues which are useful for synthesis of non-natural oligonucleotide analogues which exhibit excellent anti-sense or anti-gene activity and in vivo stability.

This invention relates to novel oligonucleotide analogues which have one or more of said bicyclonucleoside moieties.

Further, this invention relates to novel modified bicyclonucleoside analogues which exhibit anti-AIDS activity.

BACKGROUND ART

Oligonucleotides having excellent anti-sense or anti-gene activities and in vivo stability have been expected to be useful medicaments.

However, it is well known that natural oligonucleotides are rapidly decomposed by various nucleases in the blood or cells.

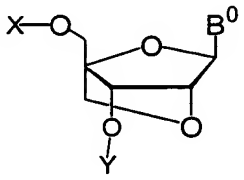
To solve these problems, numerous non-natural oligonucleotide analogues have been synthesized, and it has been tried to develop them as medicaments. For example, oligonucleotides wherein the oxygen atom binding to the phosphorus atom of the phosphodiester linkage is substituted by a sulfur atom, a methyl group, or a boron atom, are known. Further, oligonucleotides whose sugar and/or base moieties are chemically modified are also known.

More concretely, ISIS Co. has developed a thioate oligonucleotide, ISIS2922, as a therapeutic agent for retinitis infected by human cytomegalovirus and this has been sold as Vitravene (trade name) in the United States.

Any non-natural oligonucleotide analogues described above, however, have not been fully satisfactory due to their

insufficient potency of anti-sense or anti-gene activity, (*i.e.*, ability to form complementary strands with mRNA or DNA) and stability to various nucleases, and due to side effects caused by non-selective binding to various proteins in vivo. Thus it has been desired to develop non-natural oligonucleotide analogues having more potent anti-sense or anti-gene activities, in vivo stability, and fewer side effects.

Compounds having a dioxabicyclo[2,2,1]heptane moiety which is related to that of the present invention and which is shown below are described in WO98/39352. These compounds differ from the compounds of the present invention in the substituent at the 3′ position of ribose. Further, it has not been known that these compounds exhibit anti-AIDS activity.



wherein B^O indicates pyrimidine or purine nucleic acid base or their analogues, X and Y are the same or different and each represents a hydrogen atom, an alkyl group, an alkenyl group, an alkynyl group, a cycloalkyl group, an aralkyl group, an aryl group, an acyl group or a silyl group.

An objective of the present invention is to provide novel bicyclonucleoside analogues which are useful for synthesis of non-natural oligonucleotides which exhibit excellent anti-sense or anti-gene activity and in vivo stability.

An objective of the present invention is also to provide novel oligonucleotide analogues having 1 or more relevant bicyclonucleoside moieties.

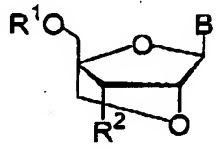
Furthermore, another objective of the present invention is to provide novel bicyclonucleoside analogues having anti-

AIDS activity.

The present inventors have performed painstaking research to complete these objectives, and found that novel bicyclonucleoside analogues having a 2'-O,4'-C-methylene moiety are important intermediate compounds to synthesize non-natural oligonucleotides which have excellent anti-sense or anti-gene activity, as well as in vivo stability. Further, the present inventors found that the novel oligonucleotide analogues having one or more of said bicyclonucleoside moieties exhibit excellent anti-sense or anti-gene activity as well as in vivo stability. Further, said bicyclonucleoside analogues have excellent anti-AIDS activity. Thus the present inventors have completed the present invention.

DISCLOSURE OF THE INVENTION

1) The novel bicyclonucleoside analogues are the compounds represented by the general formula (1) or their pharmaceutically acceptable salts,



(1)

[Wherein R^1 is the same or different, and each represents a hydrogen atom, a protecting group for a hydroxy group in nucleic acid synthesis, a phosphoric acid group, a phosphoric acid group protected with a protecting group in nucleic acid synthesis, or a group represented by the formula $-P(R^{4a})R^{4b}$ (wherein R^{4a} and R^{4b} are the same or different and each represents a hydroxy group, a hydroxy group protected with a protecting group in nucleic acid synthesis, a mercapto group, a mercapto group protected with a protecting group in nucleic acid synthesis, an amino group, an amino group protected with a protecting group in nucleic acid synthesis, an alkoxy group

having 1-6 carbon atoms, an alkylthio group having 1-6 carbon atoms, a cyanoalkoxy group having 1-7 carbon atoms, or an amino group substituted by an alkyl group having 1-6 carbon atoms),

R^2 represents an azido group, an amino group, or a group represented by the formula -NH-R^3 (wherein R^3 is the same or different and each represents a protecting group for an amino group in nucleic acid synthesis, a phosphoric acid group, a phosphoric acid group protected with a protecting group in nucleic acid synthesis, or a group represented by the formula -P(R^{4a})R^{4b} (wherein R^{4a} and R^{4b} are the same or different and each represents a hydroxy group, a hydroxy group protected with a protecting group in nucleic acid synthesis, a mercapto group, a mercapto group protected with a protecting group in nucleic acid synthesis , an amino group, an amino group protected with a protecting group in nucleic acid synthesis, an alkoxy group having 1-6 carbon atoms, an alkylthio group having 1-6 carbon atoms, a cyanoalkoxy group having 1-7 carbon atoms or an amino group substituted by an alkyl group having 1-6 carbon atoms).

B represents a purine-9-yl group or a 2-oxo-1,2-dihydropyrimidin-1-yl group each of which is optionally substituted with 1 or more substituents selected from the following α group].

(α Group)

a hydroxy group,
a hydroxy group protected with a protecting group in nucleic acid synthesis,
an alkoxy group having 1-6 carbon atoms,
a mercapto group,
a mercapto group protected with a protecting group in nucleic acid synthesis,
an alkylthio group having 1-6 carbon atoms,
an amino group,
an amino group protected with a protecting group in

nucleic acid synthesis,
an amino group substituted by an alkyl group having 1-6 carbon atoms,
an alkyl group having 1-6 carbon atoms, and
halogen atom.

Among the compounds of the present invention, preferred compounds are as follows;

2) Compounds wherein R^1 represents a hydrogen atom, an aliphatic acyl group, an aromatic acyl group, a silyl group, a methyl group substituted by 1 to 3 aryl groups, or a methyl group substituted by 1 to 3 aryl groups wherein the aryl rings are substituted by a lower-alkyl group, a lower-alkoxy group, a halogen atom or a cyano group.

3) Compounds wherein R^1 represents a hydrogen atom, a silyl group, a methyl group substituted by 1 to 3 aryl groups, or a methyl group substituted by 1 to 3 aryl groups wherein the aryl rings are substituted by a lower-alkyl group, a lower-alkoxy group, a halogen atom or a cyano group.

4) Compounds wherein R^1 represents a hydrogen atom, a trimethylsilyl group, a t-butyldimethylsilyl group, a t-butyldiphenylsilyl group, a benzyl group, a triphenylmethyl group, a 4-methoxybenzyl group, a 4-methoxyphenyldiphenylmethyl group, a 4,4'-dimethoxytriphenylmethyl group, or a 4,4',4''-trimethoxytriphenylmethyl group.

5) Compounds wherein R^2 represents an azido group, an amino group, or a group represented by the formula -NH-R^3 (wherein R^3 represents an aliphatic acyl group, an aromatic acyl group, a methyl group substituted by 1 to 3 aryl groups, a methyl group substituted by 1 to 3 aryl groups wherein the aryl rings are substituted by lower-alkyl group, lower-alkoxy group, halogen atom, or cyano group, a silyl group, a

phosphoroamidite group, a phosphonyl group, a phosphoric acid group or a phosphoric acid group substituted by a protecting group in nucleic acid synthesis),

6) Compounds wherein R^2 represents an azido group, an amino group, or a group represented by the formula -NH-R^3 (wherein R^3 represents an acetyl group, a trifluoroacetyl group, a benzoyl group, a benzyl group, a p-methoxybenzyl group, a tert-butyldiphenylsilyl group, a group represented by the formula $-P(OC_2H_4CN)(NCH(CH_3)_2)$, a group represented by a formula $-P(OCH_3)(NCH(CH_3)_2)$, a phosphonyl group, or a 2-chlorophenyl- or a 4-chlorophenylphosphoric acid group),

7) Compounds wherein R^2 represents an azido group or an amino group,

8) Compounds where B represents 6-aminopurin-9-yl (*i.e.*, adeninyl), 6-amino-purin-9-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 2,6-diaminopurin-9-yl wherein one or both amino group(s) are protected with a protecting group in nucleic acid synthesis, 2-amino-6-chloropurin-9-yl, 2-amino-6-chloropurin-9-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 2-amino-6-fluoropurin-9-yl, 2-amino-6-fluoropurine-9-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 2-amino-6-bromopurine-9-yl, 2-amino-6-bromopurin-9-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 2-amino-6-hydroxypurin-9-yl (*i.e.*, guaninyl), 2-amino-6-hydroxypurin-9-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 6-amino-2-methoxypurin-9-yl, 6-amino-2-methoxypurin-9-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 6-amino-2-chloropurin-9-yl, 6-amino-2-chloropurin-9-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 6-amino-2-

fluoropurin-9-yl, 6-amino-2-fluoropurin-9-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 2,6-dimethoxypurin-9-yl, 2,6-dichloropurin-9-yl, 6-mercaptopurin-9-yl, 6-mercaptopurine-9-yl wherein the mercapto group is protected with a protecting group in nucleic acid synthesis, 2-oxo-4-amino-1,2-dihydropyrimidin-1-yl (*i.e.*, cytosinyl), 2-oxo-4-amino-1,2-dihydropyrimidin-1-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 4-amino-2-oxo-5-fluoro-1,2-dihydropyrimidin-1-yl, 4-amino-2-oxo-5-fluoro-1,2-dihydropyrimidin-1-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 4-amino-2-oxo-5-chloro-1,2-dihydropyrimidin-1-yl, 4-amino-2-oxo-5-chloro-1,2-dihydropyrimidin-1-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 2-oxo-4-methoxy-1,2-dihydropyrimidin-1-yl, 2-oxo-4-mercapto-1,2-dihydropyrimidin-1-yl, 2-oxo-4-mercapto-1,2-dihydropyrimidin-1-yl wherein the mercapto group is protected with a protecting group in nucleic acid synthesis , 2,4-dihydroxypyrimidin-1-yl (*i.e.*, uracilyl), 2,4-dihydroxy-5-methylpyrimidin-1-yl (*i.e.*, thyminyl), 4-amino-5-methyl-2-oxo-1,2-dihydropyrimidin-1-yl, or 4-amino-5-methyl-2-oxo-1,2-dihydropyrimidin-1-yl group wherein the amino group is protected with a protecting group in nucleic acid synthesis ,

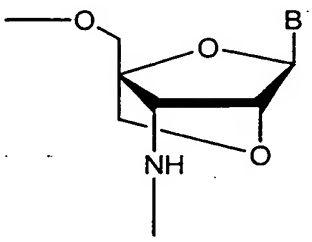
9) Compounds wherein B represents 6-benzoylaminopurin-9-yl, adeninyl, 2-benzoylamino-6-hydroxypurin-9-yl, guaninyl, 2-oxo-4-benzoylamino-1,2-dihydropyrimidin-1-yl, cytosinyl, uracilyl or thyminyl.

Compounds in which R^1 is selected from the above 2) to 4) and R^2 is selected from the above 5) to 7) and B is selected from the above 8) or 9) are also preferred. Compounds where R^1 is selected from 2), R^2 is selected from 5), and B is selected from 8), and where R^1 is selected from 3), R^2 is selected from 6), and B is selected from 8), and where R^1 is selected from 4), R^2 is selected from 6), and B is selected from 9) are

particularly preferred.

The novel oligonucleotide analogues of the present invention are:-

(1) Oligonucleotide analogues and pharmacetuically acceptable salts thereof having 1 or more structural units of formula (1a), provided that when an oligonucleotide has two or more structural units of formula (1a), each B is the same or different.



(1a)

[wherein,

B represents a purin-9-yl group or a 2-oxo-1,2-dihydropyrimidin-1-yl group which may be substituted with substituents selected from α group below.].

(α group)

a hydroxy group,

a hydroxy group protected with a protecting group in nucleic acid synthesis ,

an alkoxy group having 1-6 carbon atoms,

a mercapto group,

a mercapto group protected with a protecting group in nucleic acid synthesis,

an alkylthio group having 1-6 carbon atoms,

an amino group,

an amino group protected with a protecting group in nucleic acid synthesis,

an amino group substituted by alkyl group having 1-6 carbon atoms,

an alkyl group having 1-6 carbon atoms, and

halogen atom.

Herein, "oligonucleotide analogues" represent non-natural oligonucleotides in which nucleoside units of a natural oligonucleotide are substituted with 1 or more nucleoside moieties having above structure (1a). For example, the oligonucleotide analogues involve modified sugar derivatives, thioate derivatives in which phosphodiester-binding sites are thioated, esters in which the phosphoric acid moiety is esterified, and amide derivatives in which an amino group in a purine base is amidated as other nucleoside or nucleotide moieties.

Among the novel oligonucleotide analogues of the present invention, preferred oligonucleotide analogues are compounds and their pharmaceutically acceptable salts , wherein:

2) B is a 6-aminopurin-9-yl group (*i.e.*, an adeninyl group), a 6-aminopurin-9-yl group wherein the amino group is protected with a protecting group in nucleic acid synthesis, a 2,6-diaminopurin-9-yl group, a 2-amino-6-chloropurin-9-yl group, a 2-amino-6-chloropurin-9-yl group wherein the amino group is protected with a protecting group in nucleic acid synthesis, a 2-amino-6-fluoropurin-9-yl group, a 2-amino-6-fluoropurin-9-yl group wherein the amino group is protected with a protecting group in nucleic acid synthesis, a 2-amino-6-bromopurin-9-yl group, a 2-amino-6-bromopurin-9-yl group wherein the amino group is protected with a protecting group in nucleic acid synthesis, a 2-amino-6-hydroxypurin-9-yl group (*i.e.*, a guaninyl group), a 2-amino-6-hydroxypurin-9-yl group wherein the amino group is protected with a protecting group in nucleic acid synthesis, a 2-amino-6-hydroxypurin-9-yl group wherein the amino group and hydroxy group are protected with a protecting group in nucleic acid synthesis, a 6-amino-2-methoxypurin-9-yl group, a 6-amino-2-chloropurin-9-yl group, a 6-amino-2-fluoropurin-9-yl group, a 2,6-dimethoxypurin-9-yl group, a 2,6-dichloropurin-9-yl group, a 6-mercaptopurin-9-yl group, a 2-oxo-4-amino-1,2-dihydropyrimidin-1-yl group (*i.e.*,

a cytosinyl group), a 2-oxo-4-amino-1,2-dihydropyrimidin-1-yl group wherein the amino group is protected with a protecting group in nucleic acid synthesis, a 2-oxo-4-amino-5-fluoro-1,2-dihydropyrimidin-1-yl group, a 2-oxo-4-amino-5-fluoro-1,2-dihydropyrimidin-1-yl group wherein the amino group is protected with a protecting group in nucleic acid synthesis, a 4-amino-2-oxo-5-chloro-1,2-dihydropyrimidin-1-yl group, a 2-oxo-4-methoxy-1,2-dihydropyrimidin-1-yl group, a 2-oxo-4-mercapto-1,2-dihydropyrimidin-1-yl group, a 2-oxo-4-hydroxy-1,2-dihydropyrimidin-1-yl group (*i.e.*, an uracinyl group), a 2-oxo-4-hydroxy-5-methyl-1,2-dihydropyrimidin-1-yl group (*i.e.*, a thyminyl group), a 4-amino-5-methyl-2-oxo-1,2-dihydropyrimidin-1-yl group (*i.e.*, a 5-methylcytosinyl group), or a 4-amino-5-methyl-2-oxo-1,2-dihydropyrimidin-1-yl group wherein the amino group is protected with a protecting group in nucleic acid synthesis, and

3) compounds and their pharmaceutically acceptable salts in which B is a 6-benzoylaminopurin-9-yl group, an adeninyl group, a 2-isobutyrylamino-6-hydroxypurin-9-yl group, a guaninyl group, a 2-oxo-4-benzoylamino-1,2-dihydropyrimidin-1-yl group, a cytosinyl group, a 2-oxo-5-methyl-4-benzoylamino-1,2-dihydropyrimidin-1-yl group, a 5-methylcytosinyl group, an uracinyl group or a thyminyl group.

The "protecting group for a hydroxy group in nucleic acid synthesis " in the definition of above R^1 has no limitation, as far as the protecting group can protect the hydroxy group stably in nucleic acid synthesis. Examples of protecting groups are

"An aliphatic acyl group", for example, an alkylcarbonyl group such as formyl, acetyl, propionyl, butyryl, isobutyryl, pentanoyl, pivaloyl, valeryl, isovaleryl, octanoyl, decanoyl, 8-methylnonanoyl, 3-ethyloctanoyl, 3,7-dimethyloctanoyl, undecanoyl, tridecanoyl, hexadecanoyl, 14-methylpentadecanoyl, 13,13-dimethyltetradecanoyl, 1-methylheptadecanoyl, nonadecanoyl, eicosanoyl and henicosanoyl, a carboxylated

alkylcarbonyl group such as succinoyl, glutaroyl, and adipoyl, a halogeno-lower-alkylcarbonyl group such as chloroacetyl, dichloroacetyl, trichloroacetyl, and trifluoroacetyl, a lower-alkoxy-lower-alkylcarbonyl group such as methoxyacetyl, and an unsaturated alkylcarbonyl group such as (E)-2-methyl-2-butenoyl;

"an aromatic acyl group",for example, an arylcarbonyl group such as benzoyl, α-naphthoyl, and β-naphthoyl, a halogenoarylcarbonyl group such as 2-bromobenzoyl, 4-chlorobenzoyl, a lower-alkylated-arylcarbonyl group such as 2,4,6-trimethylbenzoyl, and 4-toluoyl, a lower-alkoxylated arylcarbonyl group such as 4-anisoyl, a carboxylated arylcarbonyl group such as 2-carboxybenzoyl, 3-carboxybenzoyl, and 4-carboxybenzoyl, a nitrated arylcarbonyl group such as 4-nitrobenzoyl, and 2-nitrobenzoyl; a lower-alkoxycarbonylated arylcarbonyl group such as 2-(methoxycarbonyl)benzoyl, an arylated arylcarbonyl group such as 4-phenylbenzoyl;

"a tetrahydropyranyl or tetrahydrothiopyranyl group" such as tetrahydropyran-2-yl, 3-bromotetrahydropyran-2-yl, 4-methoxytetrahydropyran-4-yl, tetrahydrothiopyran-2-yl, and 4-methoxytetrahydrothiopyran-4-yl;

"a tetrahydrofuranyl or a tetrahydrothiofuranyl group" such as tetrahydrofuran-2-yl, and tetrahydrothiofuran-2-yl;

"silyl groups", for example, a tri-lower-alkyl silyl group such as trimethylsilyl, triethylsilyl, isopropyldimethylsilyl, t-butyldimethylsilyl, methyldiisopropylsilyl, methyldi-t-butylsilyl, and triisopropylsilyl, a tri-lower-alkyl silyl group substituted by 1-2 aryl groups such as diphenylmethylsilyl, t-butyldiphenylsilyl, diphenylisopropylsilyl, and phenyldiisopropylsilyl;

"a lower-alkoxymethyl group" such as methoxymethyl, 1,1-dimethyl-1-methoxymethyl, ethoxymethyl, propoxymethyl, isopropoxymethyl, butoxymethyl, and t-butoxymethyl;

"a lower-alkoxylated lower-alkoxymethyl group" such as 2-methoxyethoxymethyl;

"a halogeno-lower-alkoxymethyl group" such as 2,2,2-trichloroethoxymethyl, and bis(2-chloroethoxy)methyl;

"a lower-alkoxylated ethyl group" such as 1-ethoxyethyl, and 1-(isopropoxy)ethyl;
"a halogenated ethyl group" such as 2,2,2-trichloroethyl;
"a methyl group substituted by 1 to 3 aryl groups" such as benzyl, α-naphthylmethyl, β-naphthylmethyl, diphenylmethyl, triphenylmethyl, α-naphthyldiphenylmethyl, and 9-anthrylmethyl;
"a methyl group substituted by 1 to 3 aryl groups wherein the aryl ring is substituted by lower-alkyl, lower-alkoxy, halogen or cyano groups" such as 4-methylbenzyl, 2,4,6-trimethylbenzyl, 3,4,5-trimethylbenzyl, 4-methoxybenzyl, 4-methoxyphenyldiphenylmethyl, 4,4'-dimethoxytriphenylmethyl, 4,4',4''-trimethoxytriphenylmethyl, 2-nitrobenzyl, 4-nitrobenzyl, 4-chlorobenzyl, 4-bromobenzyl, and 4-cyanobenzyl;
"a lower-alkoxycarbonyl group" such as methoxycarbonyl, ethoxycarbonyl, t-butoxycarbonyl, and isobutoxycarbonyl;
"a lower-alkoxycarbonyl group substituted by a halogen atom or a tri-lower-alkylsilyl group" such as 2,2,2-trichloroethoxycarbonyl, and 2-trimethylsilylethoxycarbonyl,
"an alkenyloxycarbonyl group" such as vinyloxycarbonyl, and aryloxycarbonyl; "an aralkyloxycarbonyl group wherein the aryl ring may be substituted by 1 or 2 lower-alkoxy or nitro groups" such as benzyloxycarbonyl, 4-methoxybenzyloxycarbonyl, 3,4-dimethoxydibenzyloxycarbonyl, 2-nitrobenzyloxycarbonyl, and 4-nitrobenzyloxycarbonyl.

Preferred protecting groups are an aliphatic acyl group, an aromatic acyl group, a methyl group substituted by 1 to 3 aryl groups, a methyl group substituted by 1 to 3 aryl groups wherein the aryl ring is substituted by lower-alkyl, lower-alkoxy group, halogen atom or cyano group, or a silyl group. More preferred protecting groups are acetyl group, benzoyl group, benzyl group, p-methoxybenzyl group, dimethoxytrityl group, monomethoxytrityl group or tert-butyldiphenylsilyl group.

Protecting groups in nucleic acid synthesis described as "a phosphoric acid group protected with a protecting group in

nucleic acid synthesis" in the above definition of R^1 and R^3 have no limitation, as far as the protecting group can protect phosphoric acid group in nucleic acid synthesis. Examples of the protecting groups are "a lower-alkyl group" such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, s-butyl, tert-butyl, n-pentyl, isopentyl, 2-methylbutyl, neopentyl, 1-ethylpropyl, n-hexyl, isohexyl, 4-methylpentyl, 3-methylpentyl, 2-methylpentyl, 1-methylpentyl, 3,3-dimethylbutyl, 2,2-dimethylbutyl, 1,1-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 2,3-dimethylbutyl, and 2-ethylbutyl; "a cyanated lower-alkyl group" such as 2-cyanoethyl, and 2-cyano-1,1-dimethylethyl; "an ethyl group substituted by a silyl group" such as 2-methyldiphenylsilylethyl, 2-trimethylsilylethyl, and 2-triphenylsilylethyl; "a halogenated lower-alkyl group" such as 2,2,2-trichloroethyl, 2,2,2-tribromoethyl, 2,2,2-trifluoroethyl, and 2,2,2-trichloro-1,1-dimethylethyl; "a lower-alkenyl group" such as ethenyl, 1-propenyl, 2-propenyl, 1-methyl-2-propenyl, 1-methyl-1-propenyl, 2-methyl-1-propenyl, 2-methyl-2-propenyl, 2-ethyl-2-propenyl, 1-butenyl, 2-butenyl, 1-methyl-2-butenyl, 1-methyl-1-butenyl, 3-methyl-2-butenyl, 1-ethyl-2-butenyl, 3-butenyl, 1-methyl-3-butenyl, 2-methyl-3-butenyl, 1-ethyl-3-butenyl, 1-pentenyl, 2-pentenyl, 1-methyl-2-pentenyl, 2-methyl-2-pentenyl, 3-pentenyl, 1-methyl-3-pentenyl, 2-methyl-3-pentenyl, 4-pentenyl, 1-methyl-4-pentenyl, 2-methyl-4-pentenyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, and 5-hexenyl; "a cycloalkyl group" such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, norbornyl, and adamantyl;
"a cyanated lower-alkenyl group" such as 2-cyanobutenyl;
"an aralkyl group" such as benzyl, α-naphthylmethyl, β-naphthylmethyl, indenylmethyl, phenanthrenylmethyl, anthracenylmethyl, diphenylmethyl, triphenylmethyl, 1-phenethyl, 2-phenethyl, 1-naphthylethyl, 2-naphthylethyl, 1-phenylpropyl, 2-phenylpropyl, 3-phenylpropyl, 1-naphthylpropyl, 2-naphthylpropyl, 3-naphthylpropyl, 1-phenylbutyl, 2-phenylbutyl, 3-phenylbutyl, 4-phenylbutyl, 1-

naphthylbutyl, 2-naphthylbutyl, 3-naphthylbutyl, 4-naphthylbutyl, 1-phenylpentyl, 2-phenylpentyl, 3-phenylpentyl, 4-phenylpentyl, 5-phenylpentyl, 1-naphthylpentyl, 2-naphthylpentyl, 3-naphthylpentyl, 4-naphthylpentyl, 5-naphthylpentyl, 1-phenylhexyl, 2-phenylhexyl, 3-phenylhexyl, 4-phenylhexyl, 5-phenylhexyl, 6-phenylhexyl, 1-naphthylhexyl, 2-naphthylhexyl, 3-naphthylhexyl, 4-naphthylhexyl, 5-naphthylhexyl, and 6-naphthylhexyl, "an aralkyl group wherein the aryl ring is substituted by nitro group, and/or halogen atom" such as 4-chlorobenzyl, 2-(4-nitrophenyl)ethyl, o-nitrobenzyl, 4-nitrobenzyl, and 2,4-dinitrobenzyl, 4-chloro-2-nitrobenzyl;
"an aryl group" such as phenyl, indenyl, naphthyl, phenanthrenyl, and anthracenyl;
"an aryl group substituted by lower-alkyl group, halogen atom, and/or nitro group" such as 2-methylphenyl, 2,6-dimethylphenyl, 2-chlorophenyl, 4-chlorophenyl, 2,4-dichlorophenyl, 2,5-dichlorophenyl, 2-bromophenyl, 4-nitrophenyl, 4-chloro-2-nitrophenyl.

Preferred protecting groups are "a lower alkyl group", "a lower-alkyl group substituted by a cyano group", "an aralkyl group", "an aralkyl group wherein the aryl ring is substituted by nitro group and/or halogen atom", or "an aryl group substituted by lower-alkyl group, halogen atom, and/or nitro group".

More preferred groups are a 2-cyanoethyl group, a 2,2,2-trichloroethyl group, a benzyl group, a 2-chlorophenyl group or a 4-chlorophenyl group.

"Alkyl groups having 1-6 carbon atoms" in the definition of the above α group are, for example, straight or branched chain alkyl groups having 1-6 carbon atoms such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, s-butyl, tert-butyl, pentyl, and hexyl. Preferred groups are alkyl groups having 1-4 carbon atoms, and more preferred alkyl groups are alkyl groups having 1-2 carbon atoms, and the most preferred group is a methyl group.

The "protecting group for an amino group in nucleic acid synthesis" described in the definition of R^2 above has no limitation, as far as it can protect amino groups in nucleic acid synthesis. These protecting groups are,
"An aliphatic acyl group"
for example, an alkylcarbonyl group such as formyl, acetyl, propionyl, butyryl, isobutyryl, pentanoyl, pivaloyl, valeryl, isovaleryl, octanoyl, decanoyl, 8-methylnonanoyl, 3-ethyloctanoyl, 3,7-dimethyloctanoyl, undecanoyl, tridecanoyl, hexadecanoyl, 14-methylpentadecanoyl, 13,13-dimethyltetradecanoyl, 1-methylheptadecanoyl, nonadecanoyl, eicosanoyl and henicosanoyl; a carboxylated-alkylcarbonyl group such as succinoyl, glutaroyl, and adipoyl; a halogeno-lower-alkylcarbonyl group such as chloroacetyl, dichloroacetyl, trichloroacetyl, and trifluoroacetyl; a lower-alkoxy-lower-alkylcarbonyl group such as methoxyacetyl, an unsaturated-alkylcarbonyl group such as (E)-2-methyl-2-butenoyl;
"An aromatic acyl group", for example, an arylcarbonyl group such as benzoyl, α-naphthoyl, and β-naphthoyl; a halogeno-arylcarbonyl group such as 2-bromobenzoyl, and 4-chlorobenzoyl; a lower-alkylated-arylcarbonyl group such as 2,4,6-trimethylbenzoyl, and 4-toluoyl;
a lower-alkoxylated-arylcarbonyl group such as 4-anisoyl;
a carboxylated-arylcarbonyl group such as 2-carboxybenzoyl, 3-carboxybenzoyl, and 4-carboxybenzoyl; a nitrated-arylcarbonyl group such as 4-nitrobenzoyl, and 2-nitrobenzoyl; a lower-alkoxycarbonylated-arylcarbonyl group such as 2-(methoxycarbonyl)benzoyl, an arylated-arylcarbonyl group such as 4-phenylbenzoyl;
"a lower-alkoxycarbonyl group" such as methoxycarbonyl, ethoxycarbonyl, t-butoxycarbonyl, and isobutoxycarbonyl;
"a lower-alkoxycarbonyl group substituted by halogen atom or tri-lower-alkylsilyl group" such as 2,2,2-trichloroethoxycarbonyl, and 2-trimethylsilylethoxycarbonyl;
"an alkenyloxycarbonyl group" such as vinyloxycarbonyl, and

aryloxycarbonyl;

"an aralkyloxycarbonyl group wherein the aryl ring may be substituted by 1-2 lower-alkoxy or nitro groups" such as benzyloxycarbonyl, 4-methoxybenzyloxycarbonyl, 3,4-dimethoxybenzyloxycarbonyl, 2-nitrobenzyloxycarbonyl and 4-nitrobenzyloxycarbonyl.

Among these, preferred groups are "an aliphatic acyl group", "an aromatic acyl group", or "an aralkyloxycarbonyl group wherein the aryl ring may be substituted by 1-2 lower-alkoxy or nitro groups".

More preferred groups are "an aliphatic acyl group" or "an aralkyloxycarbonyl group wherein the aryl ring may be substituted by 1-2 lower-alkoxy or nitro groups".

A particularly preferred group is a trifluoroacetyl group or benzyloxycarbonyl group.

"Phosphoramidite group" described above represents a group of formula $-P(OR^{3a})(NR^{3b})$ (wherein R^{3a} represents an alkyl group having 1-6 carbon atoms or cyanoalkyl group having 1-7 carbon atoms, while R^{3b} represents an alkyl group having 1-6 carbon atoms).

Preferred groups are those represented by the formula $-P(OC_2H_4CN)(NCH(CH_3)_2)$ or the formula $-P(OCH_3)(NCH(CH_3)_2)$.

"Halogen atom" described in the above definition of the α group is a fluorine, chlorine, bromine, or iodine atom, and preferred atoms are fluorine or chlorine atoms.

"Alkyl group having 1-6 carbon atoms" described in the above definition of R^{4a}, R^{4b} and α group is, for example, a straight or branched chain alkyl group having 1-6 carbon atoms, such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, s-butyl, tert-butyl, pentyl and hexyl. Preferred groups are methyl or ethyl groups.

"Hydroxy group protected with a protecting group in

nucleic acid synthesis" described in the above definition of R^{4a}, R^{4b} and α group is a similar group to that described previously in the "protecting group for a hydroxy group in nucleic acid synthesis" in the above definition of R^1. Preferred groups are "an aliphatic acyl group" and "an aromatic acyl group", and the most preferred group is a benzoyl group.

"Mercapto group protected with a protecting group in nucleic acid synthesis" described in the above definitions of R^{4a}, R^{4b} and α group is, for example, "a disulfide-forming group", for example an alkylthio group such as methylthio, ethylthio and tert-butylthio, and an arylthio group such as benzylthio, in addition to the groups described in the "protecting group for a hydroxy group in nucleic acid synthesis" in the definition of R^1.

Among these, preferred groups are "an aliphatic acyl group" or "an aromatic acyl group", and the most preferred group is a benzoyl group.

The "amino group protected with a protecting group in nucleic acid synthesis" described in the above definitions of R^{4a}, R^{4b} and α group is a similar group to those described in the "protecting group for an amino group in nucleic acid synthesis", which has been already described in the definition of R^2. Preferred groups are "aliphatic acyl groups" or "aromatic acyl groups", and the most preferred group is a benzoyl group.

"Alkoxy group having 1-6 carbon atoms" described in the above definitions of R^{4a}, R^{4b} and α group is, for example, a straight or branched chain alkoxy group having 1-6 carbon atoms, such as methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, isobutoxy, s-butoxy, tert-butoxy, pentyloxy, and hexyloxy. Preferred groups are methoxy or ethoxy groups.

"Alkylthio group having 1-6 carbon atoms" described in the above definitions of R^{4a}, R^{4b} and α group is, for example, a methylthio, ethylthio, propylthio, isopropylthio, butylthio, isobutylthio, s-butylthio, tert-butylthio, pentylthio or hexylthio group. Preferred groups are methylthio or ethylthio groups.

"Alkylamino group substituted by an alkyl group having 1-6 carbon atoms" described in the above definitions of R^{4a}, R^{4b} and α group is, for example, a methylamino, ethylamino, propylamino, isopropylamino, butylamino, isobutylamino, s-butylamino, tert-butylamino, pentylamino, hexylamino, dimethylamino, diethylamino, dipropylamino, diisopropylamino, dibutylamino, diisobutylamino, di(s-butyl)amino, di(tert-butyl)amino, dipentylamino, or dihexylamino group. Preferred groups are methylamino, ethylamino, dimethylamino or diethylamino groups.

"Cyanoalkoxy group having 1-7 carbon atoms" described in the above definition of R^{4a} and R^{4b} is, for example, a cyanomethoxy, cyanoethoxy, cyanopropyloxy, cyanobutyloxy, cyanopentyloxy, or cyanohexyloxy group, and the preferred group is a 2-cyanoethoxy group.

"pharmaceutically acceptable salts thereof" described above indicates the salts of the oligonucleoside analogues (1) and the oligonucleotide analogues having the above chemical structure (1a). Among these salts, preferred salts are, for example, metal salts such as alkali metal salts, e.g., sodium salts, potassium salts, lithium salts; alkaline earth metal salts, e.g. calcium salts and magnesium salts; aluminium salts, iron salts, zinc salts, copper salts, nickel salts and cobalt salts; amine salts such as inorganic salts, e.g. ammonium salts; organic salts, e.g., t-octylamine salts, dibenzylamine salts, morpholine salts, glucosamine salts, phenylglycine alkyl ester salts, ethylenediamine salts, N-methylglucamine salts, guanidine

salts, diethylamine salts, triethylamine salts, dicyclohexylamine salts, N,N'-dibenzylethylenediamine salts, chloroprocaine salts, procaine salts, diethanolamine salts, N-benzyl-phenethylamine salts, piperazine salts, tetramethylammonium salts and tris(hydroxymethyl)aminomethane salts; inorganic acid salts such as hydrohalogenic acid salts, e.g., hydrofluoric acid salts, hydrochloric acid salts, hydrobromic acid salts and hydroiodic acid salts; nitric acid salts, perchloric acid salts, sulfuric acid salts and phosphoric acid salts; organic acid salts such as lower alkanesulfonic acid salts, e.g., methanesulfonic acid salts, trifluoromethanesulfonic acid salts and ethanesulfonic acid salts; arylsulfonic acid salts, e.g., benzenesulfonic acid salts and p-toluenesulfonic acid salts; acetic acid salts, malic acid salts, fumaric acid salts, succinic acid salts, citric acid salts, tartaric acid salts, oxalic acid salts and maleic acid salts; and amino acid salts such as glycine salts, lysine salts, arginine salts, ornithine salts, glutamic acid salts and aspartic acid salts.

Among these salts, sodium salt, potassium salt and triethylamine salt are preferred for oligonucleotide analogues containing nucleoside structure (1a), and the free form of nucleoside is preferred for nucleoside analogues (1).

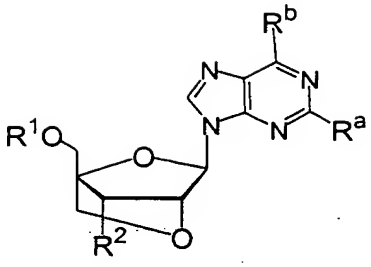
Nucleoside analogues (1) and oligonucleotide analogues having the above structure (1a) in the present invention absorb or adsorb water to form hydrates when they are left in atmosphere. These salts are included in the present invention.

Nucleoside analogues (1) and oligonucleotide analogues involving the above structure (1a) in the present invention absorb certain solvents to form solvates. These salts are included in the present invention.

Some typical examples of compound (1) of the present invention can be exemplified by Tables 1 and 2.

Abbreviations used in Table 1 and Table 2 are as follows; Bn: a benzyl group, Bz: a benzoyl group, Me: a methyl group, PMBn: a p-methoxybenzyl group, MMTr: a 4-methoxytriphenylmethyl group, DMTr: a 4,4'-dimethoxytriphenylmethyl group, TMTr: a 4,4'4''-trimethoxytriphenylmethyl group, TMS: a trimethylsilyl group, TBDMS: a tert-butyldimethylsilyl group, TBDPS: a tert-butyldiphenylsilyl group.

Table 1.



(1')

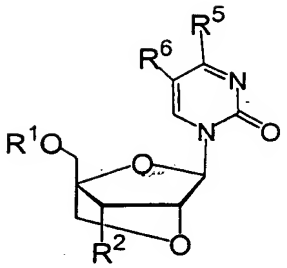
Exemplification Compound number.	R^1	R^2	R^a	R^b
1-1	H	NH_2	H	H
1-2	H	NH_2	H	OH
1-3	H	NH_2	H	SH
1-4	H	NH_2	H	NH_2
1-5	H	NH_2	H	OMe
1-6	H	NH_2	F	H
1-7	H	NH_2	F	NH_2
1-8	H	NH_2	Cl	H
1-9	H	NH_2	Cl	NH_2
1-10	H	NH_2	Cl	Cl
1-11	H	NH_2	Br	H
1-12	H	NH_2	Br	NH_2
1-13	H	NH_2	OH	H
1-14	H	NH_2	OH	OH
1-15	H	NH_2	OH	NH_2
1-16	H	NH_2	OMe	OMe
1-17	H	NH_2	OMe	NH_2
1-18	H	NH_2	NH_2	H
1-19	H	NH_2	NH_2	F
1-20	H	NH_2	NH_2	Cl

1-21	H	NH_2	NH_2	Br
1-22	H	NH_2	NH_2	OH
1-23	H	NH_2	NH_2	NH_2
1-24	H	NH_2	NH_2	OMe
1-25	H	N_3	H	H
1-26	H	N_3	H	OH
1-27	H	N_3	H	SH
1-28	H	N_3	H	NH_2
1-29	H	N_3	H	OMe
1-30	H	N_3	F	H
1-31	H	N_3	F	NH_2
1-32	H	N_3	Cl	H
1-33	H	N_3	Cl	NH_2
1-34	H	N_3	Cl	Cl
1-35	H	N_3	Br	H
1-36	H	N_3	Br	NH_2
1-37	H	N_3	OH	H
1-38	H	N_3	OH	OH
1-39	H	N_3	OH	NH_2
1-40	H	N_3	OMe	OMe
1-41	H	N_3	OMe	NH_2
1-42	H	N_3	NH_2	H
1-43	H	N_3	NH_2	F
1-44	H	N_3	NH_2	Cl
1-45	H	N_3	NH_2	Br
1-46	H	N_3	NH_2	OH
1-47	H	N_3	NH_2	NH_2
1-48	H	N_3	NH_2	OMe
1-49	H	N_3	H	NHBz
1-50	H	NH_2	H	NHBz
1-51	H	N_3	Cl	NHBz

1-52	H	N_3	OH	NHBz
1-53	H	N_3	OMe	NHBz
1-54	H	N_3	NHBz	H
1-55	H	N_3	NHBz	Cl
1-56	H	N_3	NHBz	OH
1-57	H	NH_2	NHBz	OH
1-58	H	N_3	NHBz	NHBz
1-59	H	N_3	NHBz	OMe
1-60	Bn	N_3	H	NHBz
1-61	Bn	N_3	$NHCOCH(CH_3)_2$	OH
1-62	PMBn	N_3	H	NHBz
1-63	PMBn	N_3	$NHCOCH(CH_3)_2$	OH
1-64	MMTr	N_3	H	NHBz
1-65	MMTr	N_3	$NHCOCH(CH_3)_2$	OH
1-66	DMTr	N_3	H	NHBz
1-67	DMTr	N_3	$NHCOCH(CH_3)_2$	OH
1-68	TMTr	N_3	H	NHBz
1-69	TMTr	N_3	$NHCOCH(CH_3)_2$	OH
1-70	TMS	N_3	H	NHBz
1-71	TMS	N_3	$NHCOCH(CH_3)_2$	OH
1-72	TBDMS	N_3	H	NHBz
1-73	TBDMS	N_3	$NHCOCH(CH_3)_2$	OH
1-74	TBDPS	N_3	H	NHBz
1-75	TBDPS	N_3	NHBz	OH
1-76	Bn	NH_2	H	NHBz
1-77	Bn	NH_2	$NHCOCH(CH_3)_2$	OH
1-78	PMBn	NH_2	H	NHBz
1-79	PMBn	NH_2	$NHCOCH(CH_3)_2$	OH
1-80	MMTr	NH_2	H	NHBz
1-81	MMTr	NH_2	$NHCOCH(CH_3)_2$	OH
1-82	DMTr	NH_2	H	NHBz

1-83	DMTr	NH_2	$NHCOCH(CH_3)_2$	OH
1-84	TMTr	NH_2	H	NHBz
1-85	TMTr	NH_2	$NHCOCH(CH_3)_2$	OH
1-86	TMS	NH_2	H	NHBz
1-87	TMS	NH_2	$NHCOCH(CH_3)_2$	OH
1-88	TBDMS	NH_2	H	NHBz
1-89	TBDMS	NH_2	$NHCOCH(CH_3)_2$	OH
1-90	TBDPS	NH_2	H	NHBz
1-91	TBDPS	NH_2	$NHCOCH(CH_3)_2$	OH

Table 2.



(1")

Exemplification Compound number.	R^1	R^2	R^5	R^6
2-1	H	NH_2	H	H
2-2	H	NH_2	Cl	H
2-3	H	NH_2	OH	H
2-4	H	NH_2	OH	Me
2-5	H	NH_2	SH	H
2-6	H	NH_2	NH_2	H
2-7	H	NH_2	NH_2	F
2-8	H	NH_2	NH_2	Cl
2-9	H	NH_2	NH_2	Me
2-10	H	NH_2	OMe	H
2-11	H	N_3	H	H
2-12	H	N_3	Cl	H
2-13	H	N_3	OH	H
2-14	H	N_3	OH	Me
2-15	H	N_3	SH	H
2-16	H	N_3	NH_2	H
2-17	H	N_3	NH_2	F
2-18	H	N_3	NH_2	Cl
2-19	H	N_3	NH_2	Me

2-20	H	N_3	OMe	H
2-21	H	N_3	NHBz	H
2-22	H	NH_2	NHBz	H
2-23	H	N_3	NHBz	F
2-24	H	N_3	NHBz	Cl
2-25	H	N_3	NHBz	Me
2-26	Bn	N_3	OH	H
2-27	Bn	N_3	OH	Me
2-28	Bn	N_3	NHBz	H
2-29	PMBn	N_3	OH	H
2-30	PMBn	N_3	OH	Me
2-31	PMBn	N_3	NHBz	H
2-32	MMTr	N_3	OH	H
2-33	MMTr	N_3	OH	Me
2-34	MMTr	N_3	NHBz	H
2-35	DMTr	N_3	OH	H
2-36	DMTr	N_3	OH	Me
2-37	DMTr	N_3	NHBz	H
2-38	TMTr	N_3	OH	H
2-39	TMTr	N_3	OH	Me
2-40	TMTr	N_3	NHBz	H
2-41	TMS	N_3	OH	H
2-42	TMS	N_3	OH	Me
2-43	TMS	N_3	NHBz	H
2-44	TBDMS	N_3	OH	H
2-45	TBDMS	N_3	OH	Me
2-46	TBDMS	N_3	NHBz	H
2-47	TBDPS	N_3	OH	H
2-48	TBDPS	N_3	OH	Me
2-49	TBDPS	N_3	NHBz	H
2-50	Bn	NH_2	OH	H

2-20	H	N_3	OMe	H
2-21	H	N_3	NHBz	H
2-22	H	NH_2	NHBz	H
2-23	H	N_3	NHBz	F
2-24	H	N_3	NHBz	Cl
2-25	H	N_3	NHBz	Me
2-26	Bn	N_3	OH	H
2-27	Bn	N_3	OH	Me
2-28	Bn	N_3	NHBz	H
2-29	PMBn	N_3	OH	H
2-30	PMBn	N_3	OH	Me
2-31	PMBn	N_3	NHBz	H
2-32	MMTr	N_3	OH	H
2-33	MMTr	N_3	OH	Me
2-34	MMTr	N_3	NHBz	H
2-35	DMTr	N_3	OH	H
2-36	DMTr	N_3	OH	Me
2-37	DMTr	N_3	NHBz	H
2-38	TMTr	N_3	OH	H
2-39	TMTr	N_3	OH	Me
2-40	TMTr	N_3	NHBz	H
2-41	TMS	N_3	OH	H
2-42	TMS	N_3	OH	Me
2-43	TMS	N_3	NHBz	H
2-44	TBDMS	N_3	OH	H
2-45	TBDMS	N_3	OH	Me
2-46	TBDMS	N_3	NHBz	H
2-47	TBDPS	N_3	OH	H
2-48	TBDPS	N_3	OH	Me
2-49	TBDPS	N_3	NHBz	H
2-50	Bn	NH_2	OH	H

2-51	Bn	NH_2	OH	Me
2-52	Bn	NH_2	NHBz	H
2-53	PMBn	NH_2	OH	H
2-54	PMBn	NH_2	OH	Me
2-55	PMBn	NH_2	NHBz	H
2-56	MMTr	NH_2	OH	H
2-57	MMTr	NH_2	OH	Me
2-58	MMTr	NH_2	NHBz	H
2-59	DMTr	NH_2	OH	H
2-60	DMTr	NH_2	OH	Me
2-61	DMTr	NH_2	NHBz	H
2-62	TMTr	NH_2	OH	H
2-63	TMTr	NH_2	OH	Me
2-64	TMTr	NH_2	NHBz	H
2-65	TMS	NH_2	OH	H
2-66	TMS	NH_2	OH	Me
2-67	TMS	NH_2	NHBz	H
2-68	TBDMS	NH_2	OH	H
2-69	TBDMS	NH_2	OH	Me
2-70	TBDMS	NH_2	NHBz	H
2-71	TBDPS	NH_2	OH	H
2-72	TBDPS	NH_2	OH	Me
2-73	TBDPS	NH_2	NHBz	H

Among the compounds listed in these Tables, preferred compounds are as follows (Exemplification compound numbers): 1-3, 1-4, 1-7, 1-9, 1-10, 1-16, 1-17, 1-19, 1-20, 1-21, 1-22, 1-23, 1-27, 1-28, 1 to 31, 1 to 33, 1 to 34, 1-40, 1-41, 1-43, 1-44, 1-45, 1-46, 1-47, 1-49, 1-50, 1-56, 1-57, 1-82, 1-83, 2-3, 2-4, 2-5, 2-6, 2-7, 2-8, 2-9, 2-10, 2-13, 2-14, 2-15, 2-16, 2-17, 2-18, 2-19, 2-20, 2-21, 2-22, 2-48, 2-59, 2-60, and 2-61.

More preferred compounds are as follows (Exemplification

compound numbers):

1-4, 1-22, 1-28, 1-46, 1-49, 1-50, 1-56, 1-57, 1-82, 1-83, 2-3, 2-4, 2-6, 2-13, 2-14, 2-16, 2-21, 2-22, 2-48, 2-59, 2-60, and 2-61.

Particularly preferred compounds are as follows (Exemplification compound numbers):

2-4: 3'-amino-3'-deoxy-2'-O,4'-C-methylene-5-methyluridine,

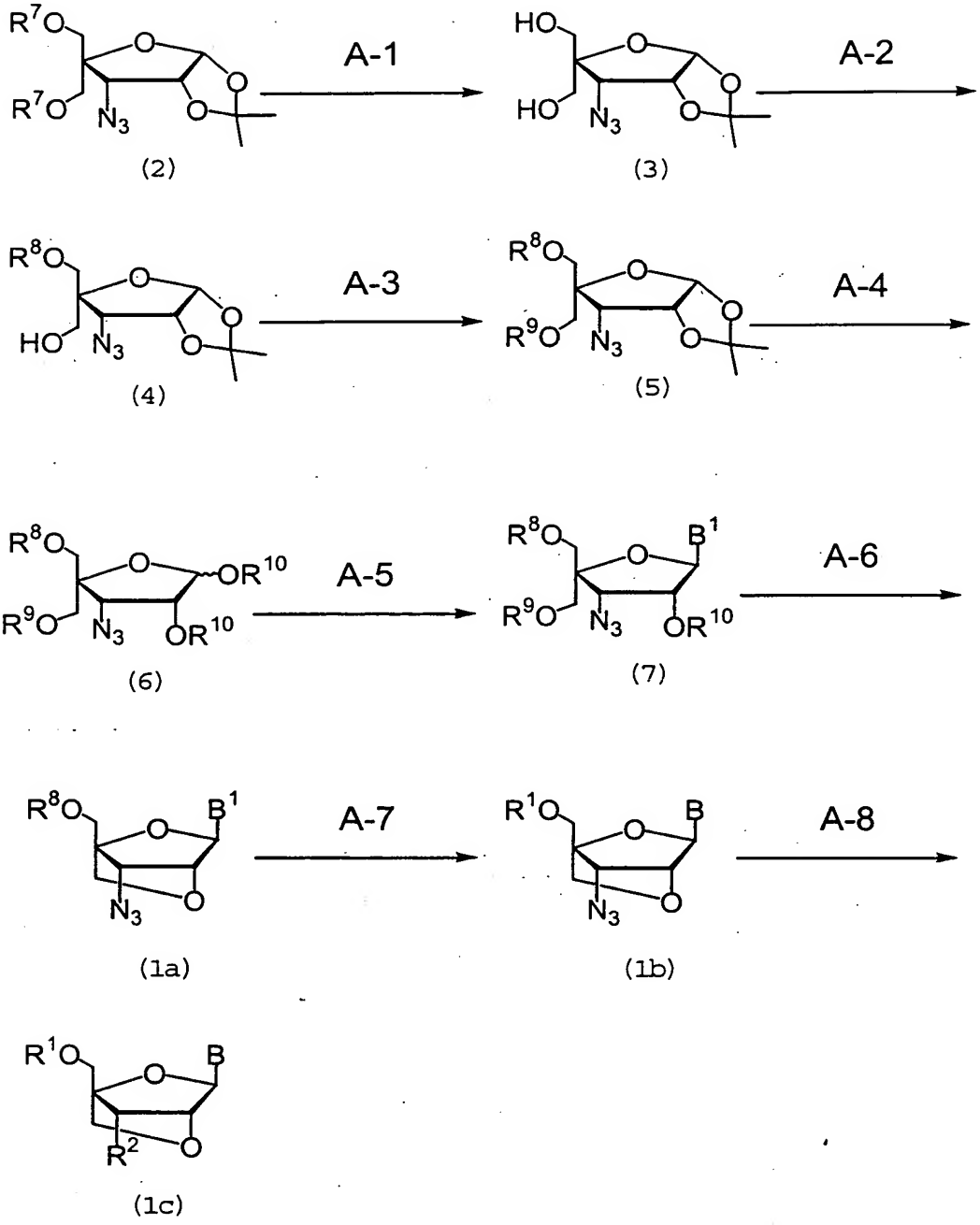
2-14: 3'-azido-3'-deoxy-2'-O,4'-C-methylene-5-methyluridine,

2-36: 3'-azido-3'-deoxy-5'-O-(4,4'-dimethoxytrityl)-2'-O,4'-C-methylene-5-methyluridine,

2-48: 3'-azido-5'-O-tert-butyldiphenylsilyl-3'-deoxy-2'-O,4'-C-methylene-5-methyluridine and

2-60: 3'-amino-3'-deoxy-5'-O-(4,4'-dimethoxytrityl)-2'-O,4'-C-methylene-5-methyluridine.

The compounds of the present invention can be synthesized in accordance with method A described below.


A-1
A-2
A-3
A-4
A-5
A-6
A-7
A-8
(2)
(3)
(4)
(5)
(6)
(7)
(1a)
(1b)
(1c)

In the processes described above, R^1, R^2 and B are as defined previously.

R^7 represents a protecting group for a hydroxy group, and preferred groups are aromatic acyl groups, for example, aryl carbonyl groups such as benzoyl, α-naphthoyl, and β-naphthoyl; lower-alkylated-arylcarbonyl groups such as 2,4,6-trimethylbenzoyl, and 4-toluoyl, and arylated-arylcarbonyl groups such as 4-phenylbenzoyl. The most preferred group is a benzoyl group.

R^8 represents a protecting group for a hydroxy group and preferred groups are
"silyl groups", for example, a tri-lower-alkylsilyl group such as trimethylsilyl, triethylsilyl, isopropyldimethylsilyl, t-butyldimethylsilyl, methyldiisopropylsilyl, methyldi-t-butylsilyl and triisopropylsilyl; and a tri-lower-alkylsilyl group substituted by 1-2 aryl groups such as diphenylmethylsilyl, t-butyldiphenylsilyl, diphenylisopropylsilyl and phenyldiisopropylsilyl;
"a methyl group substituted by 1 to 3 aryl groups" such as benzyl, α-naphthylmethyl, β-naphthylmethyl, diphenylmethyl, triphenylmethyl, α-naphthyldiphenylmethyl and 9-anthrylmethyl;
"a methyl group substituted by 1 to 3 aryl groups wherein the aryl ring is substituted by lower-alkyl, lower-alkoxy, a halogen atom or cyano group" such as 4-methylbenzyl, 2,4,6-trimethylbenzyl, 3,4,5-trimethylbenzyl, 4-methoxybenzyl, 4-methoxyphenyldiphenylmethyl, 4,4'-dimethoxytriphenylmethyl, 4,4',4''-trimethoxytriphenylmethyl, 2-nitorobenzyl, 4-nitrobenzyl, 4-chlorobenzyl, 4-bromobenzyl and 4-cyanobenzyl.

More preferred groups are a trimethylsilyl group, a t-butyldimethylsilyl group, a t-butyldiphenylsilyl group, a benzyl group, a triphenylmethyl group, a 4-methoxybenzyl group, a 4-methoxyphenyldiphenylmethyl group, a 4,4'-dimethoxytriphenylmethyl group or a 4,4',4''-trimethoxytriphenylmethyl group.

R^9 represents a leaving group and preferred groups are a lower-alkylsulfonyl group such as methanesulfonyl and ethanesulfonyl groups, a lower-alkylsulfonyl group substituted by halogen atoms such as trifluoromethanesulfonyl group, and an arylsulfonyl group such as p-toluenesulfonyl group.

Among these groups more preferred groups are methanesulfonyl group or p-toluenesulfonyl group.

R^{10} represents a protecting group for a hydroxy group and preferred groups are
"aliphatic acyl groups", for example, alkylcarbonyl groups such as formyl, acetyl, propionyl, butyryl, isobutyryl, pentanoyl, pivaloyl, valeryl, isovaleryl, octanoyl, decanoyl, 1-methylheptadecanoyl, nonadecanoyl, eicosanoyl and henicosanoyl, carboxylated alkylcarbonyl groups such as succinoyl, glutaroyl and adipoyl groups, halogeno-lower-alkylcarbonyl groups such as chloroacetyl, dichloroacetyl, trichloroacetyl and trifluoroacetyl groups, lower-alkoxy-lower-alkylcarbonyl groups such as a methoxyacetyl group, and unsaturated alkylcarbonyl groups such as a (E)-2-methyl-2-butenoyl group;
"aromatic acyl groups", for example, arylcarbonyl groups such as benzoyl, α-naphthoyl and β-naphthoyl, halogenoarylcarbonyl groups such as 2-bromobenzoyl and 4-chlorobenzoyl groups, lower-alkylated arylcarbonyl groups such as 2,4,6-trimethylbenzoyl and 4-toluoyl groups, lower-alkoxylated arylcarbonyl groups such as 4-anisoyl group, carboxylated arylcarbonyl groups such as 2-carboxybenzoyl, 3-carboxybenzoyl and 4-carboxybenzoyl groups, nitrated arylcarbonyl groups such as 4-nitrobenzoyl and 2-nitrobenzoyl groups, lower-alkoxycarbonylated arylcarbonyl groups such as 2-(methoxycarbonyl)benzoyl group,and arylated arylcarbonyl groups such as 4-phenylbenzoyl group.

Among these groups, more preferred groups are "aliphatic acyl groups" and a particularly preferred group is an acetyl group.

B^1 represents purine-9-yl or 2-oxo-1,2-dihydropyrimidin-1-yl group which may have 1 or more substituents selected from α1 group below.

(α1 group)

a hydroxy group,
a hydroxy group protected with a protecting group in nucleic acid synthesis,
an alkoxy groups having 1-6 carbon atoms,
a mercapto group,
a mercapto group protected with a protecting group in nucleic acid synthesis,
an alkylthio group having 1-6 carbon atoms,
an amino group protected with a protecting group in nucleic acid synthesis,
an amino groups substituted by an alkyl group having 1-6 carbon atoms,
an alkyl group having 1-6 carbon atoms and
halogen atoms.

Method A is a process to synthesize the compounds of formulae (1a), (1b) and (1c) from the starting compound (2) through introduction of a substitutent B and ring closure.

Here the starting compound (2) is synthesized from commercially available diacetone-D-glucose using a similar method to that described in the literature (O. T. Schmidt, Methods in Carbohydr. Chem., 4, 318 (1964); J. S. Brimacombe and O. A. Ching, Carbhyd. Res., 8, 82 (1968); T.F. Tam and B. Fraser-Reid, Can. J. Chem., 57, 2818 (1979); S. A. Suzhkov, Nucleosides & Nucleotides, 13, 2283 (1994)].

Details of each process of method A will be described below.

[Method A]

(Process A-1)

A compound (3) is prepared in this step, which comprises deprotection of a primary alcohol protecting group of starting

compound (2) in the presence of a base in an inert solvent.

The solvent employed has no limitation, insofar as the solvent is one normally used for hydrolysis, and can be water; organic solvents, for example alcohols such as methanol, ethanol and n-propanol, and ethers such as tetrahydrofuran and dioxane; or a mixture of water and the organic solvents described above. Preferred solvents are alcohols.

The base employed has no limitation unless it affects other moieties of the compound. Preferred bases are metal alkoxides such as sodium methoxide; alkali metal carbonates such as sodium carbonate, potassium carbonate and lithium carbonate; alkali metal hydroxides such as sodium hydroxide, potassium hydroxide, lithium hydroxide and barium hydroxide, or ammonia such as aqueous ammonia solution and concentrated ammonia-methanol. Preferred bases are alkali metal carbonates.

The reaction temperature and reaction time depend upon the starting material, solvent and base employed and have no limitation. Ordinarily the reaction temperature is between 0°C and 15°C and the reaction time is from 1hr to 10 hrs.

After termination of the reaction, the desired compound (3) is collected from the reaction mixture by conventional methods. For example, the reaction mixture is neutralized and concentrated, and to the residue is added water and an organic solvent immiscible with water, such as ethyl acetate. After washing with water, the organic phase including the desired compound is isolated, and dried over anhydrous sodium sulfate or the like. The desired compound is obtained by evaporation of the solvents.

The compound obtained is, if necessary, purified by conventional methods, such as recrystallization and/or silica gel column chromatography.

(Process A-2)

A compound (4) is prepared in this process which comprises reaction of compound (3) obtained in process A-1 with a hydroxy-protecting agent in the presence of a base in an inert solvent.

The solvent employed has no limitation, as far as it does not inhibit the reaction and dissolves the starting materials to some extent and is, for example, an aliphatic hydrocarbon such as hexane and heptane; an aromatic hydrocarbon such as benzene, toluene and xylene; a halogenated hydrocarbon such as methylene chloride, chloroform, carbon tetrachloride, dichloroethane, chlorobenzene and dichlorobenzene; an ester such as ethyl formate, ethyl acetate, propyl acetate, butyl acetate and diethyl carbonate; an ether such as diethyl ether, diisopropyl ether, tetrahydrofuran, dioxane, dimethoxyethane and diethylene glycol dimethyl ether; a nitrile such as acetonitrile and isobutyronitrile; an amide such as formamide, N,N-dimethylformamide, N,N-dimethylacetamide, N-methyl-2-pyrrolidone N-methyl-pyrrolidinone, and hexamethylphosphorotriamide. The preferred solvent is methylene chloride.

The base employed has no limitation, as far as it is used as a base in conventional reactions. For example, it can be an organic base such as N-methylmorpholine, triethylamine, tributylamine, diisopropylethylamine, dicyclohexylamine, N-methylpiperidine, pyridine, 4-pyrolidinopyridine, picoline, 4-(N,N-dimethylamino) pyridine, 2,6-di(tert-butyl)-4-methylpyridine, quinoline, N,N-dimethylaniline and N,N-diethylaniline. The preferred base is triethylamine.

The hydroxyl-protecting reagents employed are, for example, silyl halides such as t-butyldimethylsilyl chloride, trimethylsilyl chloride, triethylsilyl chloride, triethylsilyl bromide, triisopropylsilyl chloride, dimethylisopropylsilyl chloride, diethylisopropylsilyl chloride, t-butyldiphenylsilyl chloride, diphenylmethylsilyl chloride, and triphenylsilyl chloride; tritylhalides such as 4-methoxytriphenylmethyl chloride, 4,4'-dimethoxytriphenylmethyl chloride and 4,4',4''-trimethoxytriphenylmethyl chloride; and aralkyl halides such as benzyl chloride, benzyl bromide and p-methoxybenzyl bromide. The preferred hydroxyl-protecting reagent is t-butyldiphenylsilyl chloride.

The reaction temperature is usually between -20°C and the reflux temperature of the solvent employed. The preferred temperature is between 0°C and the reflux temperature of the solvent employed.

The reaction time depends upon mainly the reaction temperature, the starting compound, the base and the solvent employed. Ordinarily it is from 10 min to 3 days, and the preferred reaction time is from 1 hr to 24 hrs.

After the reaction is terminated, the desired compound (4) in the present reaction is collected from the reaction mixture, according to conventional methods. For example, the reaction mixture is neutralized, and water and an organic solvent immiscible with water, such as ethyl acetate, are added to the neutralized reaction mixture. After washing with water, the organic phase including the desired compound is separated, and dried over anhydrous sodium sulfate or the like. The desired compound is obtained by evaporation of the solvent.

The compound obtained is, if necessary, and particularly if a product in which R^8 is introduced to the hydroxy group at undesired positions is obtained, further purified by conventional methods, such as recrystallization and silica gel column chromatography.

(Process A-3)

A compound (5) is prepared in this process which comprises reaction of compound (4) obtained in process A-2 with a leaving-group introducing reagent in the presence of base in an inert solvent.

The solvent employed is, for example, an aliphatic hydrocarbon such as hexane, heptane, ligroin and petroleum ether; an aromatic hydrocarbon such as benzene, toluene and xylene; a halogenated hydrocarbon such as methylene chloride, chloroform, carbon tetrachloride, dichloroethane, chlorobenzene and dichlorobenzene; an ester such as ethyl formate, ethyl acetate, propyl acetate, butyl acetate and diethyl carbonate; an ether such as diethyl ether, diisopropyl

ether, tetrahydrofuran, dioxane, dimethoxyethane, and diethylene glycol dimethyl ether; a ketone such as acetone, methyl ethyl ketone and methyl isobutyl ketone, isophorone, and cyclohexanone; a nitro compound such as nitroethane and nitrobenzene; a nitrile such as acetonitrile and isobutyronitrile; an amide such as formamide, N,N-dimethylformamide, N,N-dimethylacetamide, N-methyl-2-pyrrolidone, N-methylpyrrolidinone, and hexamethylphosphorotriamide;
a sulfoxide such as sulfolane; or a pyridine.

Among these solvents, the preferred solvent is methylene chloride.

Preferred basic catalysts employed are bases such as triethylamine, pyridine and dimethylaminopyridine.

The leaving-group introducing reagent employed is, for example, an alkylsulfonyl halide such as methanesulfonyl chloride and ethanesulfonyl bromide; or an arylsulfonyl halide such as p-toluenesulfonyl chloride.

Preferred leaving-group introducing reagents are methanesulfonyl chloride and p-toluenesulfonyl chloride.

The reaction temperature depends upon the starting compound, solvent, leaving-group introducing reagent and base employed. Usually the temperature is between 0°C and 50°C, and the preferred temperature is between 10°C and 40°C.

The reaction time depends upon the starting compound, solvent, leaving-group introducing reagent and base employed. Usually the reaction time is from 10 min to 24 hrs, and the preferred reaction time is from 1 hr to 15 hrs.

After termination of the reaction, the desired compound (5) of this reaction is collected from the reaction mixture according to conventional methods. For example, the reaction mixture is neutralized and concentrated. Water and an organic solvent immiscible with water, such as ethyl acetate, are added to the residue. After washing with water, the organic phase including the desired compound is separated, dried over anhydrous sodium sulfate or the like, and then the desired compound can be obtained by evaporation of the solvents.

The compound obtained is, if necessary, purified by conventional methods, such as recrystallization, silica gel column chromatography and the like.

(Process A-4)

A compound (6) is prepared in this process which comprises reaction of compound (5) obtained in process A-3 with an acid anhydride in the presence of an acid catalyst in a solvent.

The solvent employed is, for example, an ether such as diethylether, dioxane and tetrahydrofuran; a nitrile such as acetonitrile and isobutyronitrile; an amide such as formamide, N,N-dimethylformamide, N,N-dimethyl-acetamide, N-methyl-2-pyrrolidone, N-methylpyrrolidinone and hexamethylphosphorotriamide; or an organic acid such as acetic acid. The preferred solvent is acetic acid.

The acid catalyst employed in process A-4 is, for example, an inorganic acid such as hydrochloric acid, sulfuric acid, or nitric acid. The preferred acid is sulfuric acid (particularly concentrated sulfuric acid).

The acid anhydride employed is, for example, a lower-aliphatic acid anhydride such as acetic acid anhydride, propionic acid anhydride and the like. The preferred acid anhydride is acetic anhydride.

The reaction temperature depends upon the starting compound, solvent, acid catalyst and acid anhydride employed. Usually the reaction temperature is between 0°C and 50°C, and the preferred reaction temperature is between 10°C and 40°C.

The reaction time depends upon the starting compound, solvent, acid catalyst, acid anhydride and the reaction temperature employed. Usually the reaction time is from 10 min to 12 hrs, and the preferred reaction time is from 30 min to 6 hrs.

After termination of the reaction, the desired compound (6) of this reaction is collected from the reaction mixture according to conventional methods. For example, water and an organic solvent immiscible with water, such as ethyl acetate,

is added to the reaction mixture. After washing with water, the organic phase including the desired compound is isolated, dried over anhydrous sodium sulfate or the like, and then the desired compound can be obtained by evaporation of the solvent.

The compound obtained is, if necessary, further purified by conventional methods, such as recrystallization, silica gel column chromatography and the like.

(Process A-5)

A compound of (7) is prepared in this process which comprises reaction of compound (6) obtained in process A-4 with a trimethylsilyl derivative of an optionally substituted purine or pyrimidine, which is prepared in accordance with the literature (H. Vorbrueggen, K. Krolikiewicz and B. Bennua, Chem Ber., 114, 1234-1255 (1981)), in the presence of an acid catalyst in an inert solvent.

The solvent employed is an aromatic hydrocarbon such as benzene, toluene and xylene; a halogenated hydrocarbon such as methylene chloride, chloroform, carbon tetrachloride, 1,2-dichloroethane, chlorobenzene and dichlorobenzene; a nitrile such as acetonitrile and isobutyronitrile; an amide such as formamide, N,N-dimethylformamide, N,N-dimethylacetamide, N-methyl-2-pyrrolidone, N-methyl-pyrrolidinone and hexamethylphosphorotriamide; or carbon disulfide. The preferred solvent is 1,2-dichloroethane.

The acid catalyst employed is, for example, a Lewis acid catalyst such as $AlCl_3$, $SnCl_4$, $TiCl_4$, $ZnCl_2$, BF_3 and trimethylsilyl trifluoromethanesulfonate. The preferred acid catalyst is tin tetrachloride ($SnCl_4$).

The reaction temperature depends upon the starting compound, solvent and acid catalyst employed. Usually the reaction temperature is between 0°C and 100°C, and the preferred reaction temperature is between 30°C and 80°C.

The reaction time depends upon the starting compound, solvent, acid catalyst, and reaction temperature employed. Usually the reaction time is from 1 hr to 3 days, and the

preferred reaction time is from 1 hr to 2 days.

After termination of the reaction, the desired compound (7) of this reaction is collected from the reaction mixture according to conventional methods. For example, the reaction mixture is neutralized, and water and an organic solvent immiscible with water, such as ethyl acetate or methylene chloride, is added to the resulting mixture. After washing with water, the organic phase including the desired compound is separated, dried over anhydrous sodium sulfate or the like, and then the desired compound can be obtained by evaporation of the solvent.

The compound obtained is, if necessary, purified by conventional methods, for example recrystallization, silica gel column chromatography, and the like.

(Process A-6)

A compound (1c) is prepared in this process which comprises a cyclization reaction of compound (7) obtained in process A-5 in the presence of a basic catalyst in an inert solvent.

The solvent employed has no limitation as far as it does not inhibit the reaction and it dissolves the starting compound to some extent. Preferred solvents are alcohols such as methanol, ethanol, n-propanol, isopropanol, n-butanol, isobutanol, t-butanol, isoamyl alcohol, diethylene glycol, glycerin, octanol, cyclohexanol and methyl cellosolve. The most preferred solvent is methanol.

The basic catalyst employed is, for example, an alkali metal hydroxide such as sodium hydroxide and potassium hydoxide; an alkali metal carbonate such as sodium carbonate and potassium carbonate;
an alkali metal alkoxide such as sodium methoxide and sodium ethoxide; or aqueous ammonia solution and the like. Preferred basic catalysts are alkaline metal carbonates and the most preferred basic catalyst is sodium carbonate.

The reaction temperature depends upon the starting compound, solvent, and basic catalyst employed. Usually the

reaction temperature is between 0°C and 50°C, and the preferred reaction temperature is between 10°C and 30°C.

The reaction time depends upon the starting compound, solvent, basic catalyst, and the reaction temperature employed. Usually the reaction time is from 1 hr to 3 days, and the preferred reaction time is from 3 hr to 2 days.

After termination of the reaction, the desired compound (1a) of this reaction is collected from the reaction mixture according to conventional methods. For example, the reaction mixture is concentrated, and water and an organic solvent immiscible with water, such as ethyl acetate, are added to the residue. After washing with water, the organic phase including the desired compound is separated, dried over anhydrous sodium sulfate or the like, and then the desired compound can be obtained by evaporation of the solvent.

The compound obtained is, if necessary, purified by conventional methods, for example, recrystallization, silica gel column chromatography, and the like.

(Process A-7)

A compound (1b) is prepared in this process which comprises reaction of compound (1a) obtained in process A-6 with a deprotecting agent in an inert solvent. In the case that deprotection is unnecessary, the next process can be conducted without this process.

The process of deprotection depends upon the protecting groups employed, and the deprotecting reagent has no limitation unless it has an adverse effect on the reaction. For instance, the deprotection can be carried out according to methods described in the literature of "Protective Groups in Organic Synthesis" (Theodora W. Greene, 1981, A Wiley-Interscience Publication).

When different kinds of protecting groups exist, some of these methods are appropriately combined and each of these carried out in turn.

Particularly when the protecting groups are (1) "aliphatic acyl or aromatic acyl groups", (2) "a methyl group

substituted by 1 to 3 aryl groups" or a "methyl groups substituted by 1 to 3 aryl rings wherein the aryl ring is substituted by lower-alkyl, lower-alkoxy, cyano group or halogen atom", (3) "silyl groups", the protecting groups can be deprotected with the following methods.

(1) When the protecting groups are aliphatic acyl or aromatic acyl groups, they are usually deprotected by reaction with bases in inert solvents.

The solvents employed have no limitation as far as they are usually used in hydrolysis. For instance, water; organic solvents, for example, alcohols such as methanol, ethanol, and n-propanol; ethers such as tetrahydrofuran and dioxane, or a mixture of water and above organic solvents are used. The preferred solvents are alcohols.

The bases employed have no limitation unless they affect other moieties of the compounds. Preferred bases are metal alkoxides such as sodium methoxide; alkali metal carbonates such as sodium carbonate, potassium carbonate and lithium carbonate; alkali metal hydroxides such as sodium hydroxide, potassium hydoxide, lithium hydroxide and barium hydroxide; or ammonia such as aqueous ammonia solutions and concentrated ammonium-ethanol. Preferred bases are alkali metal carbonates.

The reaction temperature and the reaction time depend upon the starting compound, solvent, base employed. Usually the reaction temperature is between 0°C and 150°C and the reaction time is from 1 hr. to 10 hrs. in order to suppress production of by-products.

After termination of the reaction, the desired compound (1b) of this reaction is collected from the reaction mixture according to conventional methods. For example, the reaction mixture is concentrated, and water and an organic solvent immiscible with water, such as ethyl acetate, are added to the residue. After washing with water, the organic phase including the desired compound is separated, dried over anhydrous sodium sulfate or the like, and then the desired compound can be obtained by evaporation of the solvent.

The compound obtained is, if necessary, purified by

conventional methods, for example recrystallization, silica gel column chromatography and the like.

(2) In the case that the protecting group is "a methyl group substituted by 1 to 3 aryl groups" or " a methyl group substituted by 1 to 3 aryl groups wherein aryl ring is substituted by lower-alkyl, lower-alkoxy group, halogen atom or a cyano group", deprotection is carried out by a reducing reagent in an inert solvent.

Preferred solvents employed are alcohols such as methanol, ethanol and isopropanol; ethers such as diethyl ether, tetrahydrofuran and dioxane; aromatic hydrocarbons such as toluene, benzene and xylene; aliphatic hydrocarbons such as hexane and cyclohexane; esters such as ethyl acetate and propyl acetate; organic acids such as acetic acid; or mixtures of these organic solvents and water.

The reducing reagents employed have no limitation if they are usually used in catalytic reactions. Preferred reducing agents are palladium-carbon, Raney nickel, platinum oxide, platinum black, rhodium-aluminium oxide, triphenylphosphine-rhodium chloride and palladium-barium sulfate.

The reaction pressure has no limitation. Usually this process is performed under 1 to 10 atmosphere.

The reaction temperature is between 0°C and 60°C, and the preferred reaction temperature is between 20°C and 40°C.

The reaction time is from 10 min. to 24 hrs. and the preferred reaction time is from 1 to 3 hrs.

After termination of the reaction, the desired compound (1b) of this reaction is collected from the reaction mixture according to conventional methods. For example, the reducing reagent is removed, and water and an organic solvent immiscible with water, such as ethyl acetate, are added to the reaction mixture. After washing with water, the organic phase including the desired compound is separated, dried over anhydrous sodium sulfate or the like, and then the desired compound can be obtained by evaporation of the solvent.

The compound obtained is, if necessary, further purified by conventional methods, for example recrystallization, silica gel column chromatography and the like.

When the protecting group is "a methyl group substituted by 3 aryl groups", *i.e.*, when the protecting group is a trityl group, deprotection can also be carried out using an acid.

In this case, the following solvents are used, for example,
aromatic hydrocarbons such as benzene, toluene and xylene; halogenated hydrocarbons such as methylene chloride, chloroform, carbon tetrachloride, 1,2-dichloroethane, chlorobenzene and dichlorobenzene; alcohols such as methanol, ethanol, isopropanol and tert-butanol; nitriles such as acetonitrile and isobutyronitrile; amides such as formamide, N,N-dimethyl formamide, N,N-dimethylacetamide, N-methyl-2-pyrrolidone, N-methyl-pyrrolidinone, and hexamethylphosophorotriamide; and organic acids such as acetic acid. Preferred solvents are organic acids (particularly acetic acid) and alcohols (particularly tert-butanol).

The preferred acid to use is acetic acid or trifluoroacetic acid.

The reaction temperature is between 0°C and 60°C, and the preferred reaction temperature is between 20°C and 40°C.

The reaction time is from 10 min to 24 hrs and the preferred reaction time is from 1 to 3 hrs.

After termination of the reaction, the desired compound (1b) of this reaction is collected from the reaction mixture according to conventional methods. For example, the reaction mixture is neutralized, and water and an organic solvent immiscible with water, such as ethyl acetate, are added to the resulting mixture. After washing with water, the organic phase including the desired compound is separated, dried over anhydrous sodium sulfate or the like, and then the desired compound can be obtained by evaporation of the solvent.

The compound obtained is, if necessary, further purified by conventional methods, for example recrystallization, silica gel column chromatography and the like.

(3) In the case that the protecting group is "a silyl group", the protecting group is usually deprotected by treatment with compounds which produce fluorine anion, such as tetrabutylammonium fluoride, hydrofluoric acid, hydrofluoric acid-pyridine, and potassium fluoride, or organic acids such as acetic acid, methanesulfonic acid, para-toluenesulfonic acid, trifluoroacetic acid, and trifluoromethanesulfonic acid, or inorganic acids such as hydrochloric acid.

When the protecting group is deprotected with fluorine anion, the reaction is, in some cases, accelerated by addition of an organic acid such as formic acid, acetic acid or propionic acid.

The solvents used have no limitation as far as they do not inhibit the reaction and they dissolve the starting materials to some extent. However, preferred solvents are ethers such as diethyl ether, diisopropylether, tetrahydrofuran, dioxane, dimethoxyethane and diethylene glycol dimethyl ether; nitriles such as acetonitrile and isobutyronitrile; water; organic acids such as acetic acid, and mixtures of these solvents described above.

The reaction temperature is between 0°C and 100°C, and the preferred reaction temperature is between 20°C to 70°C.

The reaction time is from 5 min. to 48 hrs. and the preferred reaction time is from 1 to 24 hrs.

After termination of the reaction, the desired compound (1b) of this reaction is collected from the reaction mixture according to conventional methods. For example, the solvents are evaporated and then the compound is purified by silica gel column chromatography.

(Process A-8)

A compound (1c) is prepared in this process which comprises catalytic reduction of the azido group in compound (1b) obtained in process A-7 to an amino group in the presence of hydrogen and a catalyst in an inert solvent and, if necessary, protection of the amino group.

The solvents employed have no limitation as far as they do not have an adverse effect on this reaction. Preferred solvents are alcohols such as methanol, ethanol and isopropanol; ethers such as diethylether, tetrahydrofuran and dioxane; aromatic hydrocarbons such as toluene, benzene and xylene; aliphatic hydrocarbons such as hexane and cyclohexane; esters such as ethyl acetate and propyl acetate; amides such as formamide, dimethylformamide, dimethylacetamide, N-methyl-2-pyrrolidone and hexamethylphosphorotriamide; aliphatic acids such as formic acid and acetic acid; water; or mixtures of these solvents described above.

The catalysts employed have no limitation if they are usually used in catalytic reductions. Preferred catalysts are palladium on carbon, palladium black, Raney nickel, platinum oxide, platinum black, rhodium-aluminium oxide, triphenylphosphine-rhodium chloride, palladium-barium sulfate.

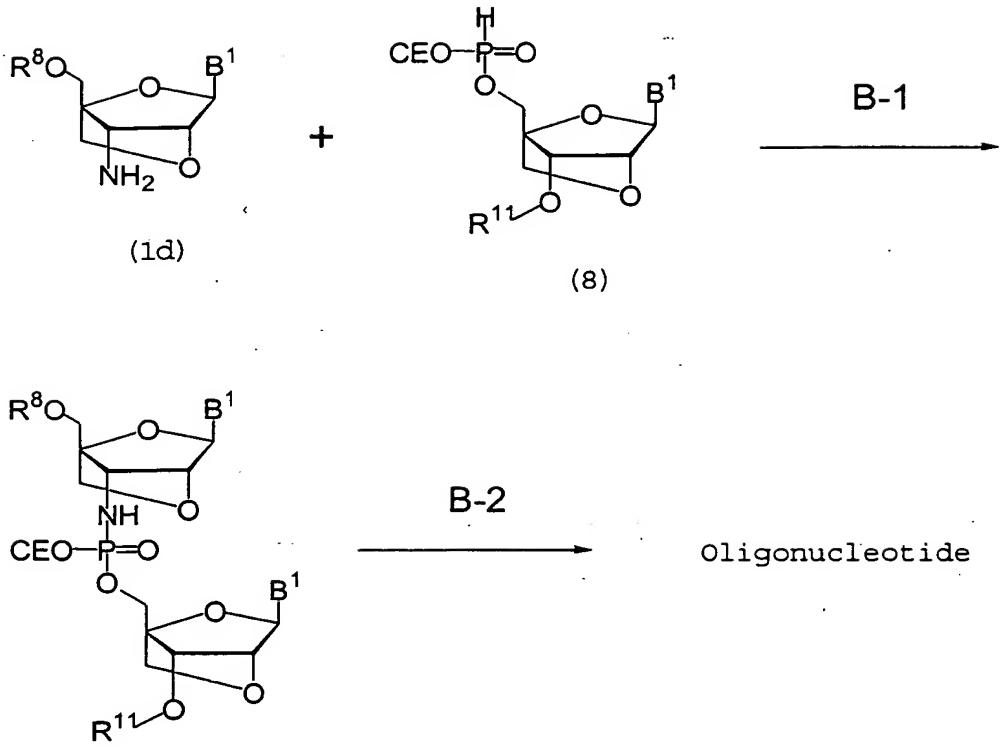
The reaction pressure has no limitation, but is usually between 1 and 10 atmospheres.

The reaction temperature and reaction time depends upon the starting compound, solvent, and catalyst employed. Usually the reaction temperature is between 0°C and 100°C (preferred reaction temperature is between 20°C and 40°C), and the reaction time is from 5 min. to 48 hrs. (preferred reaction time is from 30 min. to 10 hrs.).

After termination of the reaction, the desired compound (1c) of this reaction is collected from the reaction mixture according to conventional methods. For example, the desired compound can be obtained through removal of the catalysts by filtration and by evaporation of solvent from the filtrate.

If desired, the amino group can be protected in accordance with the methods described in the above literature (Protective Groups in Organic Synthesis).

A N3'-P5' type oligonucleotide analogue of this invention in which the nitrogen atom at 3' position and the oxygen atom at 5' position are combined through phosphoric acid can be prepared using compound (1d) of this invention according to method B described below.



In the processes described above, B^1 and R^8 are as defined previously. However B^1 in the formula (1d) and B^1 in the formula (8) may be the same or different.

R^{11} represents a resin such as succinyl Controlled Pore Glass or Tentagel, which is usually employed for the synthesis of oligonucleotides.

CEO represents a 2-cyanoethoxy group.

Each process of method B will be described below in detail.

(Process B-1)

A compound (9) is prepared in this process which comprises an oxidative phosphorylation coupling reaction of compound (1d) with compound (8). This process is performed as described in the literature (1) (Nucleic Acids Research, Vol. 23, No. 14, pp. 2661-2668, 1995).

The hydroxy group at the 5' position of the compound

(1d) is protected in compound (1c) in Process A-8, and if an amino group exists in the base B, said amino group of compound (1d) is protected.

Further, the compound (8) can be prepared from the compound (1c) obtained in "Process A-8", in accordance with the literature (1).

(Process B-2)

This process is to produce an oligonucleotide from compound (9) obtained in the "Process B-1".

The process comprises deprotection of the hydroxyl-protecting group R^8 of compound (9) by a procedure of process A-7, phosphorylation in accordance with the literature (1), reaction with compound (1d) in a method similar to that described in the Process B-1, followed by repetition of these reactions to give the desired oligonucleotide.

The sequence length of the oligonucleotides obtained is usually 2-50 nucleoside units, and the preferred length is 10-30 nucleoside units.

The oligonucleotide analogues obtained are resistant to various nucleases. Thus they remain in the body for a long time after administration. Further, the oligonucleotide analogues, for instance, form stable double strands with mRNA, and inhibit biosynthesis of proteins which contribute to pathogenesis, or inhibit transcription to mRNA by forming triplets with the DNA double strands in genomes, or inhibit proliferation of viruses.

Thus the oligonucleotide analogues of the present invention can supress specified genome functions, and are expected to be therapeutic agents used for the treatment of diseases, such as anti-neoplasm agents, anti-viral agents, or the like.

Non-oral formulations or liposome formulations of the

oligonucleotide analogues of this invention can be prepared, for instance, by addition of conventional adjuvants such as buffers and/or stabilizers. The nucleotide analogues may be blended with conventional pharmaceutical carriers to prepare ointments, creams, liquids or plasters.

Their doses are different according to the symptoms of the disease, the age of the patient, and the route of administration. For instance, the lowest dose of the oligonucleotide analogue is 0.001 mg/kg of the body weight (preferably 0.01 mg/kg of the body weight), and the highest dose is 100 mg/kg of the body weight (preferably 10 mg/kg of the body weight) as a single dose. It is desirable to administer from one to several times throughout the day depending on the symptoms of the patient.

The present invention will be described below in more detail by way of the following Examples and Reference examples. However, the present invention is not limited to those examples.

Best mode for carrying out the invention

(Example 1)

3'-Azido-5'O-tert-butyldiphenylsilyl-3'-deoxy-2'-0,4'-C-methylene-5-methyluridine (Exemplification compound number 2-48)

Potassium carbonate (41 mg, 0.29 mmol) was added to a methanol solution (7 ml) of the compound obtained in Reference example 5 (200 mg, 0.27 mmol) at 0°C and the mixture was stirred for 4.5 hrs at room temperature. Further potassium carbonate (34 mg, 0.25 mmol) was added to the mixture, which was stirred for 23 hrs. After the methanol was evaporated, the residue was partitioned between ethyl acetate and water. The extract was washed with saturated aqueous sodium chloride solution. The organic phase was dried over anhydrous sodium sulfate. The solvents were evaporated and the residue was purified by silica gel column chromatography (ethyl acetate: n-hexane = 2:1) to give the title compound as colorless crystals (142 mg,

0.27 mmol, 100%).

mp 93-95°C.

IR vmax (KBr): 3169, 3047, 2956, 2888, 2859, 2117, 1696, 1275, 1109 cm^{-1}.

^1H-NMR ($CDCl_3$) δ: 1.12 (9H, s), 1.65 (3H, s), 3.78, 3.84 (2H, AB, J = 8 Hz), 3.90, 4.08 (2H, AB, J = 12.5 Hz), 4.02 (1H, s), 4.67 (1H, s), 5.67 (1H, s), 7.54 (1H, s), 7.39-7.48 (6H, m), 7.67-7.71 (4H, m), 8.46 (1H, br s).

^{13}C-NMR ($CDCl_3$) δ: 12.3, 19.5, 27.0, 58.7, 60.3, 71.4, 77.2, 78.6, 87.2, 90.1, 110.8, 128.0, 130.1, 130.2, 131.7, 132.3, 133.7, 135.1, 135.4, 149.6, 163.6.

(Example 2)

3'-Azido-3'-deoxy-2'-O,4'-C-methylene-5-methyluridine

(Exemplification compound number 2-14)

Anhydrous tetrabutylammonium fluoride (10 M in THF, 290 µl, 0.29 mmol) was added to an anhydrous tetrahydrofuran solution (5 ml) of the compound obtained in Example 1 (140 mg, 0.26 mmol) in a stream of nitrogen gas and the solution was stirred for 1 hr at room temperature. The solvent was evaporated and the residue was purified by silica gel column chromatography (ethyl acetate: n-hexane = 25:1) and the title compound was obtained as a white powder (65.7 mg, 0.22 mmol, 85%).

mp 94-96°C.

IR vmax (KBr): 3163, 3046, 2118, 1692, 1468, 1273, 1062 cm^{-1}.

^1H-NMR (CD_3OD) δ: 1.89 (3H, s), 3.76, 3.86 (2H, AB, J = 8 Hz), 3.85, 3.95 (2H, AB, J = 13 Hz), 4.03 (1H, s), 4.58 (1H, s), 5.58 (1H, s), 7.70 (1H, s).

^{13}C-NMR (CD_3OD) δ: 12.8, 57.3, 61.2, 72.4, 79.8, 88.3, 91.0, 110.8, 136.3, 151.5, 166.1.

(Example 3)

3'-Amino-3'-deoxy-2'-O,4'-C-methylene-5-methyluridine

(Exemplification compound number 2-4)

An ethanol solution (3 ml) of the compound obtained in Example

2 (64 mg, 0.22 mmol) was added to 10% palladium-carbon (28 mg) suspended in anhydrous tetrahydrofuran solution (5 ml) in a stream of hydrogen gas, and the mixture was stirred for 0.5 hr at room temperature. The reaction mixture was filtered and the solvent of the filtrate was evaporated and the title compound was obtained as a white powder (59 mg, 0.22 mmol, 100%).

mp 243-246°C.

IR νmax (KBr): 3459, 3365, 1699, 1447, 1273, 1054 cm^{-1}.

^1H-NMR (C_5D_5N) δ: 1.83 (3H, s), 3.62 (1H, s), 3.92, 4.14 (2H, AB, J = 8 Hz), 4.24 (2H, s), 4.54 (1H, s), 5.97 (1H, s), 7.90 (1H, s).

^{13}C-NMR (C_5D_5N) δ: 12.8, 54.2, 57.2, 71.6, 81.4, 91.1, 109.5, 150.8, 164.3.

(Example 4)

3'-Azido-3'-deoxy-5'-0-(4,4'-dimethoxytrityl)-2'-0,4'-C-methylene-5-methyluridine (Exemplification compound number 2-36)

Dimethoxytritylchloride (415 mg, 1.22 mmol) and dimethylaminopyridine (12.5 mg, 0.10 mmol) was added to a pyridine solution (6 ml) of the compound obtained in Example 2 (300 mg, 1.02 mmol) in a stream of nitrogen gas and the solution was stirred for 20.5 hr at room temperature. Saturated aqueous sodium hydrogen carbonate solution was added to the reaction mixture and the resulting mixture was extracted with dichloromethane. The organic phase was washed with water and saturated aqueous sodium chloride solution, and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (n-hexane: ethyl acetate = 2 : 1 → 1:1) and the title compound was obtained as a pale yellow foam (462 mg, 0.78 mmol, 76%).

mp 125-128°C.

^1H-NMR ($CDCl_3$) δ: 1.66 (3H, s), 3.32, 3.65 (2H, ABq, *J* = 11 Hz), 3.78 (2H, s), 3.80 (6H, s), 4.13 (1H, s), 4.63 (1H, s), 5.67 (1H, s), 6.86 (4H, dd, *J* = 2 Hz, 9 Hz), 7.23-7.45 (9H,

m), 7.73 (1H, s), 8.04 (1H, brs).

(Example 5)

3'-Amino-3'-deoxy-5'-O-(4,4'-dimethoxytrityl)-2'-O,4'-C-methylene-5-methyluridine (Exemplification compound number 2-60)

Triphenylphosphine (94.0 ml, 0.36 mmol) was added to a pyridine solution (2.5 ml) of the compound obtained in Example 4 (110 mg, 0.18 mmol) in a stream of nitrogen and the mixture was stirred for 3.5 hr at room temperature. 28% solution of aqueous ammonia (5.5 ml) was added to the reaction mixture which was stirred for 24 hrs at room temperature. The solvent was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (chloroform : ethanol = 20 : 1) and the title compound was obtained as a pale yellow foam (462 mg, 0.78 mmol, 76%).

mp 131-134°C.

^{1}H-NMR (Pyridine-d_5) δ: 1.89 (3H, s), 3.71 (6H, s), 3.77 (1H, s), 3.84 (2H, s), 3.99, 4.10 (2H, ABq, J = 8 Hz), 4.69 (1H, s), 6.04 (1H, s), 7.03-7.87 (13H, m), 8.58 (1H, s).

(Example 6)

3'-Amino-3'-deoxy-5'-O-(4,4'-dimethoxytrityl)-2'-O,4'-C-methylene-5-methyluridinyl-(3'→5')-3'-O-(tert-butyldimethylsilyl)thymidine 2-cyanoethyl ester

A carbon tetrachloride solution (0.3 ml) of the compound obtained in Example 5 (10.0 mg, 18 μmol), and a solution of triethylamine (0.05 ml, 0.36 mmol) in acetonitrile (0.2 ml), were added to an acetonitrile solution (0.3 ml) of the compound obtained in Reference Example 6 (14.5 mg, 0.28 μmol) in a stream of nitrogen gas, and the solution was stirred for 14.5 hr at room temperature. The solvent was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (n-hexane : ethyl acetate = 1:1→0:1) and the title compound was obtained as a white powder (13.0 mg, 12.5 μmol, 71%).

mp 101-105°C.

^{31}P-NMR ($CDCl_3$) δ: 7.68, 8.24. Mass(FAB): m/z 1043(M^++H).

(Example 7)

3'-Amino-3'-deoxy-5'-O-(4,4'-dimethoxytrityl)-2'-O,4'-C-methylene-5-methyluridinyl-(3'→5')-3'-O-(tert-butyldimethylsilyl)thymidine methyl ester

A carbon tetrachloride solution (0.3 ml) of the compound obtained in Example 5 (10.0 mg, 18 μmol), and a solution of triethylamine (0.05 ml, 0.36 mmol) in acetonitrile (0.2 ml), were added to an acetonitrile solution (0.3 ml) of the compound obtained in Reference Example 7 (22.1 mg, 51 μmol) in a stream of nitrogen gas, and the solution was stirred for 18 hrs at room temperature. Water was added to the reaction mixture and the resulting mixture was extracted with ethyl acetate. The organic phase was washed with saturated aqueous sodium chloride solution and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (dichloromethane : methanol = 20:1, 30:1) and the title compound was obtained as a white powder (6.9 mg, 6.87 μmol, 39%).

mp 118-122°C.

^{31}P-NMR ($CDCl_3$) δ: 11.20, 11.30. Mass(FAB): m/z 1026(M^++Na).

(Example 8)

3'-Amino-3'-deoxy-5'-O-(4,4'-dimethoxytrityl)-2'-O,4'-C-methylene-5-methyluridinyl-(3'→5')-thymidine methyl ester

A tetrahydrofuran solution of tetrabutylammonium fluoride (1.0 M, 15 μl, 15 μmol) was added to tetrahydrofuran solution (1 ml) of the compound obtained in Example 7 (13.9 mg, 14 μmol) in a stream of nitrogen gas, and the solution was stirred for 3 hrs at room temperature. The solvent was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (ethyl acetate : ethanol = 5:1) and the title compound was obtained as a colorless powder (9.7 mg,

10.9 μmol, 78%).

mp 157-160°C.

^{31}P-NMR ($CDCl_3$) δ: 11.20, 11.30. Mass(FAB): m/z 1026(M^++Na).

(Example 9)

3'-Amino-3'-deoxy-5'-0-(4,4'-dimethoxytrityl)-2'-0,4'-C-methylene-5-methyluridinyl-(3'→5')-2'-[cyanoethoxy(diisopropylamino)phosphino]thymidine methyl ester

Tetrahydrofuran (0.2 ml) was added to an acetonitrile solution (0.6 ml) of the compound obtained in Example 8 (10.0 mg, 11 μmol) and diisopropylammonium tetrazolide (15.5 mg, 77 μmol), then 2-cyanoethyldiisopropylchlorophosphoroamidite (39.8 mg, 132 μmol), were added in a stream of nitrogen gas and the solution was stirred for 25 hrs at room temperature. The solvent was evaporated under reduced pressure. The residue compound was purified by silica gel column chromatography (ethyl acetate: triethylamine = 99:1→ethyl acetate : ethanol : triethylamine = 100 : 10 : 1). The product was reprecipitated with dichloroethane and n-hexane and the title compound was obtained as a white powder (3.8 mg, 3.5 μmol, 31%).

mp 113-116°C.

^{31}P-NMR (CD_3OD) δ: 8.67, 8.77, 9.07, 9.28, 148.53, 148.93, 148.99, 149.03.

(Example 10) Synthesis of oligonucleotide analogues

By using a DNA synthesizer (manufactured by Pharmacia Co., Gene Assembler Plus), oligonucleotides were automatically synthesized in 0.2 μmol scale. Solvents and concentrations of reagents and phosphoramidite in each process of the production are identical to those in production of natural oligonucleotides. The solvents, reagents and phosphoramidites of natural nucleosides employed were those supplied from Pharmacia. The DMTr group of Universal QCPG (0.2 μmol, manufactured by Glen Research) was deprotected with trichloroacetic acid, and the hydroxy group produced was

treated with the compound obtained in Example 9 or amidites used in the synthesis of natural nucleotides. This condensation process was repeated to obtain oligonucleotide analogues of desired sequences. The synthetic cycle was as follows;

Synthesis Cycle

1) Detritylation trichloroacetate / dichloromethane; 60 sec.
2) Coupling phosphoramidite (25 eq) tetrazole / acetonitrile; 2 min or 30 min.
3) Capping 1-methylimidazole / acetonitrile, anhydrous acetic acid / 2,4,6-collidine/acetonitrle; 36 sec.
4) Oxidation iodine / water / pyridine / acetonitrile; 60 sec.

When the compound obtained in Example 10 was reacted in the above cycle 2, the reaction time was 30 min, and when other phosphoramidites were employed, the reaction time was 2 min. After the oligonucleotide having the desired sequence was synthesized, the synthetic cycle was conducted until cycle 1 described above, the dimethoxytrityl group at the 5' position was deprotected, and then, following conventional methods, the oligomer was cut off from its supporting substance with concentrated aqueous ammonia solution, the protecting group of cyanoethyl group on the phosphorus atom was deprotected, and the protecting groups on the nucleic acid bases were deprotected.

The oligomer was purified by reverse phase HPLC and the desired oligonucleotide was obtained.

According to this method, the oligonucleotide analogue 5'-ttttttttttnt-3' (sequence number 1 in the sequence table), of which n in base number 11 was 3'-amino-3'deoxy-2'-0,4'-C-methylene-5-methyluridine (hereinafter called "oligonucleotide (1)") was obtained.
(yield 8.5 nmol, 4.3%)

The obtained oligonucleotide analogues were purified by

reverse phase HPLC (HPLC : Model 302, column manufactured by GILSON; CHEMCO CHEMCOBOND 5-ODS-H (7.8 × 300 mm); 0.1 M aqueous triethylamine acetate solution (TEAA), pH7; 10→12.5%CH_3CN / 40 min, linear gradient; 50°C; 2.5 ml/min; 254 nm), and the fraction eluted at 25.4 min was collected.

(Example 11) Synthesis of oligonucleotide analogues
By using 5'O-dimethoxytrityl-N-4-benzoyl-5-methyl-2'deoxycytidine -3'-O-(2-cyanoethyl)N,N-diisopropylphosphoramidite (manufactured by Pharmacia CO.), a nucleotide analogue having the sequence represented as 5'-tttttmtntmtmtmt-3' (sequence number 2 in the sequence table), in which m represents 5-methyl 2'-deoxycytidine and n represents 3'-amino-3'-deoxy-2'-O,4'-C-methylene-5-methyluridine, (hereinafter called "oligonucleotide (2)" was obtained (yield 7.1 nmol, 3.5%).

The modified oligonucleotide analogue which was obtained was purified with reverse phase HPLC (HPLC: Model 302, Column manufactured by GILSON; CHEMCO CHEMCOBOND 5-ODS-H (7.8 × 300 mm); 0.1 M aqueous solution of triethylamine acetate (TEAA), pH7; 10→12%CH_3CN / 40 min, linear gradient; 50°C; 2.5 ml/min; 254 nm), and the fraction eluted at 22.5 min was collected.

(Reference Example 1)
3-Azido-3-deoxy-4-hydroxymethyl-1,2-O-isopropylidene-α-D-ribofuranose
Potassium carbonate (380 mg, 2.75 mmol) and water (15 ml) were added to a methanol solution (85 ml) of 3-azido-4-benzoyloxymethyl-5-O-benzoyl-3-deoxy-1,2-O-isopropylidene-α-D-ribofuranose (4.13 g, 9.15 mmol) prepared in accordance with the literature (Surzhykov S.A., Krayevsky A.A., Nucleosides Nucleotides, 13, 2283-2305 (1994)) at 0°C, and the mixture was stirred for 4.5 hrs at 0°C. Then the reaction mixture was neutralized with 10% hydrochloric acid solution at 0°C, and the methanol was evaporated. Water was added to the residue, then, after extraction with ethyl acetate, the extracts were

washed with saturated aqueous sodium chloride solution. The organic phase was dried over anhydrous sodium sulfate. The solvent was evaporated. The white solid obtained was washed with cold n-hexane and the desired compound was obtained as a white powder (1.93 g, 7.87 mmol, 86%).

mp 113-115°C (toluene).

IR νmax (KBr): 3460, 3417, 2989, 2951, 2907, 2111 cm^{-1}.

^1H-NMR ($CDCl_3$) δ: 1.62 (3H, s), 1.35 (3H, s) 2.65 (2H, br s), 3.81, 3.65 (2H, AB, J = 12 Hz), 3.59, 4.00 (2H, AB, J = 12.5 Hz), 4.28 (1H, d, J = 5.5 Hz), 4.82 (1H, dd, J = 4 Hz, 5.5 Hz), 5.85 (1H, d, J = 4 Hz).

^{13}C-NMR ($CDCl_3$) δ: 25.7, 26.2, 61.9, 62.1, 63.2, 79.9, 87.3, 104.4, 113.6.

(Reference Example 2)

<u>3-Azido-5-O-tert-butyldiphenylsilyl-3-deoxy-4-hydroxydimethyl-1,2-O-isopropylidene-α-D-ribofuranose</u>

Triethylamine (3.5 g, 4.82 ml, 34.6 mmol) and t-butyldiphenylsilyl chloride (9.75 g, 9.22 ml, 35.46 mmol) were added to an anhydrous methylene chloride solution (73 ml) of the compound obtained in Reference Example 1 (2.56 mg, 10.5 mmol) and the solution was stirred for 24 hrs at room temperature. Then saturated aqueous sodium hydrogen carbonate solution was added to the reaction mixture, and the resulting mixture was extracted with ethyl acetate and the extracts washed with saturated aqueous sodium chloride solution. The organic phase was dried over anhydrous sodium sulfate. After the solvent was evaporated, the residue was purified by silica gel column chromatography (ethyl acetate: n-hexane = 1:6). The desired compound was obtained as a white powder (3.13 g, 6.47 mmol, 62%).

mp 99.5-100.5°C (n-hexane).

IR νmax (KBr): 3504, 2936, 2852, 2111 cm^{-1}.

^1H-NMR ($CDCl_3$) δ: 1.07 (9H, s), 1.36 (3H, s), 1.62 (3H, s), 3.62, 3.92 (2H, AB, J = 12 Hz), 4.38 (1H, d, J = 6 Hz), 4.84 (1H, dd, J = 4 Hz, 5.5 Hz), 3.82, 3.70 (2H, AB, J = 11 Hz),

4.84 (1H, dd, J = 4 Hz, 5.5 Hz), 5.86 (1H, d, J = 4 Hz), 7.36-7.44 (6H, m), 7.64-7.67 (4H, m).

^{13}C-NMR ($CDCl_3$) δ: 19.2, 26.1, 26.3, 26.8, 62.2, 62.3, 65.2, 80.4, 88.0, 104.5, 113.7, 127.7, 127.8, 129.8, 129.9, 132.7, 132.8, 135.5.

(Reference Example 3)

3-Azido-5-O-tert-butyldiphenylsilyl-3-deoxy-4-(p-toluenesulfonyloxymethyl)-1,2-O-isopropylidene-α-D-ribofuranose

Triethylamine (137 mg, 180 µl, 1.29 mmol), p-toluenesulfonyl chloride (63.3 mg, 0.33 mmol) and 4-dimethylaminopyridine (4 mg, 0.03 mmol) were added to an anhydrous methylene chloride solution (2 ml) of the compound obtained in Reference Example 2 (100 mg, 0.21 mmol) at 0°C in a stream of nitrogen gas, and the solution was stirred for 14 hrs at room temperature. Then saturated aqueous sodium hydrogen carbonate solution was added to the reaction mixture, the resulting mixture was extracted with ethyl acetate and the extracts washed with saturated sodium chloride solution. The organic phase was dried over anhydrous sodium sulfate. After the solvent was evaporated, the residue was purified by silica gel column chromatography (ethylacetate: n-hexane = 1:6). The desired compound was obtained as a white powder (130 mg, 0.20 mmol, 98%).

mp 122-124°C(ethyl acetate-n-hexane).

IR νmax (KBr): 3069, 2935, 2114, 1366, 1183, 1109 cm^{-1}.

^{1}H-NMR ($CDCl_3$) δ: 1.03 (9H, s), 1.27 (3H, s), 1.31 (3H, s), 2.41 (3H, s), 3.60, 3.72 (2H, AB, J = 10.5 Hz), 4.33, 4.40 (2H, AB, J = 10 Hz), 4.55 (1H, d, J = 5.5 Hz), 5.00 (1H, dd, J = 3.7 Hz, 5.5 Hz), 5.82 (1H, d, J = 3.7 Hz), 7.23 (2H, d, J = 8.5 Hz), 7.36-7.45 (6H, m), 7.61-7.63 (4H, m), 7.72 (2H, d, J = 8.5 Hz).

^{13}C-NMR ($CDCl_3$) δ: 19.1, 21.5, 25.9, 26.0, 26.7, 63.1, 64.7, 68.9, 80.1, 85.6, 104.4, 113.8, 127.8, 128.0, 129.6, 129.9, 132.4, 132.5, 135.4, 144.6.

(Reference Example 4)

3-Azido-5-O-tert-butyldiphenylsilyl-3-deoxy-4-(p-toluenesulfonyloxymethyl)-1,2-di-O-acetyl-D-ribofuranose

Acetic anhydride (406 mg, 375 µl, 3.98 mmol) and concentrated sulfuric acid (6.5 mg, 3.5 µl, 0.066 mmol) were added to an acetic acid solution (3.5 ml) of the compound obtained in Reference Example 3 (230 mg, 0.36 mmol) in a stream of nitrogen gas and the solution was stirred for 5 hrs at room temperature. Then ice-water was added to the reaction mixture, and after stirring for 30 min, saturated aqueous sodium chloride solution was added. The resulting mixture was extracted with ethyl acetate. The organic phase was dried over anhydrous sodium sulfate. After the solvent was evaporated, the residue was purified by silica gel column chromatography (ethyl acetate: n-hexane = 4:1). The desired compound, which is a mixture of α:β = approximately 3:7, was obtained as a colorless oil (230 mg, 0.34 mmol, 94%).

IR νmax (KBr): 3048, 2935, 2864, 2117, 1756.cm^{-1}.

^{1}H-NMR ($CDCl_3$) [β form] δ: 1.06 (9H, s), 1.83 (3H, s), 2.08 (3H, s), 2.40 (3H, s), 3.54, 3.80 (2H, AB, J = 11 Hz), 4.12, 4.26 (2H, AB, J = 10 Hz), 4.37 (1H, d, J = 5.5 Hz), 5.32 (1H, d, J = 5.5 Hz), 5.98 (1H, s), 7.29 (2H, d, J = 8 Hz), 7.37-7.46 (6H, m), 7.59-7.65 (4H, m), 7.76 (2H, d, J = 8 Hz).

[α form] δ: 1.05 (9H, s), 2.02 (3H, s), 2.13(3H, s), 2.39 (3H, s), 3.51, 3.68 (2H, AB, J = 11 Hz), 4.12, 4.21 (2H, AB, J = 10.5 Hz), 4.40 (1H, d, J = 7 Hz), 5.32 (1H, m), 6.31 (1H, d, J = 4.5 Hz), 7.25 (2H, d, J = 8.5 Hz), 7.37-7.46 (6H, m), 7.59-7.65 (4H, m), 7.70 (2H, d, J = 8.5 Hz).

^{13}C-NMR ($CDCl_3$) δ: 19.0, 19.1, 20.0, 20.6, 20.9, 21.1, 21.5, 26.6, 61.0, 63.2, 65.1, 68.4, 68.8, 72.2, 75.5, 85.4, 86.5, 93.6, 96.0, 97.3, 127.8, 127.9, 128.0, 129.6, 129.9, 130.0, 132.0, 132.3, 132.4, 135.4, 144.7, 168.5, 169.2, 169.3, 169.4.

(Reference Example 5)

2'O-Acetyl-3'-azido-5'-O-tert-butyldiphenylsilyl-3'-deoxy-4'-(p-toluenesulfonyloxymethyl)-5-methyluridine

O,O'-Bis(trimethylsilyl)thymine (240 mg, 0.93 mmol) and tin tetrachloride (253 mg, 114 µl, 0.97 mmol) were added to an anhydrous 1,2-dichloroethane solution (6 ml) of the compound obtained in Reference Example 4 (300 mg, 0.44 mmol) at 0°C in a stream of nitrogen gas, and the solution was stirred for 43 hrs at room temperature. After the reaction mixture was diluted with dichloromethane in an ice bath, saturated aqueous sodium hydrogen carbonate solution was added to the reaction mixture, which was then extracted with dichloromethane. The extracts were washed with saturated aqueous sodium chloride solution. After the organic phase was dried over anhydrous sodium sulfate, the solvent was evaporated and the residue was purified by silica gel column chromatography (ethyl acetate: n-hexane = 1:2–1:0). The desired compound was obtained as a white powder (300 mg, 0.4 mmol, 91%).

mp 158.5-159.5°C (ethyl acetate-n-hexane).

^{1}H-NMR ($CDCl_3$) δ: 1.11 (9H, s), 1.59 (3H, s), 2.15 (3H, s), 2.41 (3H, s), 3.80, 3.84 (2H, AB, J = 11.5 Hz), 4.04, 4.10 (2H, AB, J = 11 Hz), 4.47 (1H, d, J = 6 Hz), 5.53 (1H, t, J = 6.5 Hz), 5.94 (1H, d, J = 7 Hz), 7.18 (1H, s), 7.28 (2H, d, J = 7.5 Hz), 7.37-7.47 (6H, m), 7.61-7.65 (4H, m), 7.71 (2H, d, J = 7.5 Hz), 9.68 (1H, br s).

^{13}C-NMR ($CDCl_3$) δ: 11.8, 19.2, 20.9, 21.5, 26.9, 62.3, 65.9, 68.3, 74.2, 84.8, 86.1, 118.9, 127.9, 128.0, 129.7, 130.1, 131.5, 132.2, 135.2, 135.3, 135.5, 145.0, 150.4, 163.6, 169.9.

(Reference Example 6)

<u>3'-O-(tert-Butyldimethylsilyl)thymidine-5'-(2-cyanoethyl)phosphonate</u>

2-Cyanoethyltetraisopropylphosphorodiamidite (132 mg, 0.44 mmol) was added over 5 min to an acetonitrile solution (4 ml) of 3'-O-(tert-butyldimethylsilyl)thymidine (described in K.M. Fries, C. Joswing and R.F. Borch, J. Med. Chem., <u>38</u>, 2672 (1995)) (100 mg, 0.34 mmol) in a stream of nitrogen gas and the solution was stirred for 2.2 hrs at room temperature. Then, an acetonitrile solution (0.88 ml) of tetrazole (30.8

mg, 0.44 mmol) was added and the solution was stirred for 1.5 hr at room temperature. Water was added to the reaction mixture, which was extracted with dichloroethane. The organic phase was washed with saturated aqueous sodium chloride solution, and then dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (chloroform: methanol = 30:1, n-hexane:ethyl acetate=1:5→0:1). The title compound was obtained as a colorless oil (98.4 mg, 0.21 mmol, 70%).

^{1}H-NMR ($CDCl_3$) δ: 0.10 (6H, s), 0.90 (9H, s), 1.96 (3H, s), 2.16-2.28 (2H, m), 2.77-2.82 (2H, m), 4.09-4.41 (6H, m), 6.28 (1H, dd, *J* = 7 Hz, 11 Hz), 6.98 (1H, d, *J* = 720 Hz), 7.36 (1H, d, *J* = 8 Hz), 8.20(1H, brs). ^{31}P-NMR ($CDCl_3$) δ: 7.70, 8.94.

(Reference Example 7)

3'-O-(tert-butyldimethylsilyl)thymidine-5'-methylphosphonate

Chlorodiisopropylaminomethoxyphosphine (69.2 mg, 0.35 mmol) was added over 5 min to a dichloromethane solution (2 ml) of 3'-O-(tert-butyldimethylsilyl)thymidine (100 mg, 0.28 mmol) in a stream of nitrogen gas, and the solution was stirred for 1 hrs at room temperature. Then, an acetonitrile solution (2 ml) of tetrazole (56.0 mg, 0.80 mmol) was added and the solution was stirred for 40 min at room temperature. Water was added to the reaction mixture, which was extracted with dichloroethane, and the organic phase was washed with saturated aqueous sodium chloride solution and was dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (n-hexane:ethyl acetate = 1:1→0:1, n-hexane: ethyl acetate = 1:4). The title compound was obtained as a colorless oil (109 mg, 0.25 mmol, 91%).

^{31}P-NMR ($CDCl_3$) δ: 9.13, 10.07.

(Test example 1)

(Measurement of Tm for Determination of Activity of Triplet

Formation)

A sample solution of equimolar amounts of oligonucleotide (2), which forms a triplet, and a natural oligonucleotide with double stranded DNA (final concentration of each nucleotide is 1.5 μM) in 7 mM sodium phosphate buffer solution (pH 7.0) containing 140 mM KCl and 10 mM $MgCl_2$ (or a solution without 10 mM $MgCl_2$) was immersed in a boiling water bath. Then the solution was cooled slowly to room temperature over 12 hrs, and further cooled to 4°C and left at 4°C for 1 hr. The sample solution in a cell of a spectrophotometer (Du650 manufactured by Beckman Instrument Inc.) was warmed gradually from 5°C to 85°C (0.5°C/min) and the ultraviolet absorption of the sample was determined at 260 nm.

Natural oligonucleotides which have double stranded DNA with the sequence of 5'-gctaaaaagaaagagagatcg-3' (sequence number 3 in the sequence table) and its complementary strand with the sequence of 5'-cgatctctctttctttttagc-3' were used.

Further, a natural oligonucleotide which forms a triplet, with the sequence of 5'ttttttmtttmtmtmt-3', in which m is 5-methyl-2'deoxycytidine (hereinafter called as "oligonucleotide (3)") was used.

The results of Tm measurements of double stranded DNA with oligonucleotide (2) and (3) are shown in Table 3.

Table 3

	Tm (°C)	
	Oligonucleotide (2) Obtained in Example 11	Oligonucleotide (3)
With $MgCl_2$	55	44
Without $MgCl_2$	44	32

As clearly demonstrated, oligonucleotide analogues of the present invention showed higher Tm values in triplets than natural oligonucleotide analogues. This indicates that oligonucleotide analogues of the present invention showed high

activity in triplet formation.

(Test example 2)

(Determination of tolerance to nucleases)

0.2 μg of 3'-exonuclease (phosphodiesterase from Crotalus durissus (Boehringer Mannheim)) was added to 320 μl of buffer solution (50 mM Tris (pH 8.0) and 10 mM $MgCl_2$) containing various oligonucleotides (10μg) and the mixture was kept at 37°C. After a predetermined time, the enzyme activity was quenched by heating (90°C) a portion of the resulting mixture for 2 min. The remaining amount of oligonucleotide in the resulting mixture was determined by reverse phase HPLC and the change in the amount of oligonucleotide over time was determined in the presence of nucleases. The results are shown in Figure 1.

Brief Explanation of the Figure.

The Figure demonstrates the time course of changes in the amount of oligonucleotide in the presence of nucleases.

The Ordinate indicates ratio (%) of the amount of oligonucleotide remaining to the amount at 0 min.

The Abscissa indicates time (min) after the beginning of the reaction.

Oligonucleotides Employed in the Test

1. Oligonucleotide (1) obtained in Example 10.
2. The nucleotide with a sequence of 5'-ttttttttttnt-3' (sequence number 1 in the sequence table) in which n is 2'O,4-C-methylene-5-methyluridine (hereinafter called as "oligonucleotide (4)").
3. Natural oligonucleotide with a sequence of 5'-tttttttttttt-3' (sequence number 6 in the sequence table) (hereinafter called as "oligonucleotide (5)").

Oligonucleotide analogues of the present invention demonstrated remarkable nuclease resistance compared to the

natural oligonucleotide analogues. Further, the oligonucleotide analogues of this invention were shown to exert more potent resistance to nucleases than known non-natural oligonucleotide analogues.

The hybrid forming activity and anti-HIV activity of the oligonucleotide analogues of the present invention were able to be determined by using the following methods.

(Method 1)
The melting temperatures (Tm values) of the annealing products between antisense strands, which were the various oligonucleotide analogues obtained, and natural DNA- or RNA-based sense strands were measured to investigate the hybridizing ability of the oligonucleotide analogues of the present invention for complementary DNA and RNA.

Each sample solution (500 µl) with final concentrations of 100 mM sodium chloride, 10 mM sodium phosphate buffer (pH 7.2), 4 µM antisense strand, and 4 µM sense strand, respectively, was heated in a boiling water bath, and slowly cooled to room temperature over 10 hours. The sample solution in a cell chamber of a spectrophotometer (UV-2100PC, manufactured by Shimadzu Cor.,) was gradually cooled to 5° C, kept at 5°C for a further period of 20 minutes, and then the measurement was started, in a stream of nitrogen gas in order to prevent condensation of moisture. The sample temperature was raised at a rate of 0.2°C/minute until 90°C, and the ultraviolet absorption at 260 nm was measured at intervals of 0.1°C. In order to prevent changes of the sample concentration with increases in the temperature, a cell with a cover was used, and a drop of a mineral oil was applied on the surface of the sample solution during measurement.

(Method 2)
Determination of Anti-HIV Activity
Anti-HIV activities of the oligonucleotide analogues of the

present invention were determined by a similar method to that described by R. Pauwel et al. (J. Virological Method, 20, p. 309-321 (1988)). The cell precipitate was suspended in RPMI-1640 medium which did not contain serum. To the suspension was added HIV and the mixture was incubated at 37°C for 1 hour. At the end of this time the resulting mixture was washed with RPMI-1640 medium containing 10% fetal bovine serum (hereinafter called "serum medium") and centrifuged (1000 x g, 5 min). The HIV infected cell thus obtained and HIV non-infected cells were suspended in the serum medium so as to have a concentration of 4 x 10^5/ml, respectively. After 100 µl of the suspension was placed in each well of a 96-well plate for tissue culture, they were incubated for 5 days at 37°C in the presence of carbon dioxide gas without stirring. HIV infected cells and non-infected cells without test compounds were similarly incubated. After the incubation, the living cells were counted by using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and cell injury inhibitory activities of test compounds were determined. It was confirmed that Mycoplasma was not contained in the cell solution and virus solution incubated.

Inhibitory activity of cell injury in HIV non-infected cells without a test compound was expressed as 100%, and inhibitory activity of cell injury in HIV infected cells without a test compound was expressed as 0%. The concentration of the compound to inhibit cell injury by 50% (EC_{50}) was determined.

Possibility of Industrial Utilization

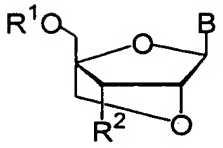
Novel bicyclonucleoside analogues of the present invention exhibit excellent anti-sense or anti-gene activities and are useful as intermediates for producing oligonucleotide analogues with in vivo stability.

Further, novel oligonucleotide analogues of the present invention are stable in vivo and useful as an anti-sense or anti-gene agents.

Moreover, novel bicyclonucleoside analogues have anti-HIV activity and are useful as a therapeutic or prophylactic agents for AIDS.

CLAIMS

1 A compound of general formula (1) or a pharmaceutically acceptable salt thereof,



(1)

[wherein, R^1 is the same or different, and each represents a hydrogen atom, a protecting group for a hydroxy group in nucleic acid synthesis, a phosphoric acid group, a phosphoric acid group protected with a protecting group in nucleic acid synthesis, or a group represented by the formula $-P(R^{4a})R^{4b}$ (wherein R^{4a} and R^{4b} are the same or different and each represents a hydroxy group, a hydroxy group protected with a protecting group in nucleic acid synthesis, a mercapto group, a mercapto group protected with a protecting group in nucleic acid synthesis, an amino group, an amino group protected with a protecting group in nucleic acid synthesis, an alkoxy group having 1-6 carbon atoms, an alkylthio group having 1-6 carbon atoms, a cyanoalkoxy group having 1-7 carbon atoms, or an amino group substituted by an alkyl group having 1-6 carbon atoms),

R^2 represents an azido group, an amino group, or a group represented by the formula $-NH-R^3$ (wherein, R^3 is the same or different and each represents a protecting group for an amino group in nucleic acid synthesis, a phosphoric acid group, a phosphoric acid group protected with a protecting group in nucleic acid synthesis, or a group represented by the formula $-P(R^{4a})R^{4b}$ (wherein R^{4a} and R^{4b} is the same or different and each represents a hydroxy group, a hydroxy group protected with a protecting group in nucleic acid synthesis, a mercapto

group, a mercapto group protected with a protecting group in nucleic acid synthesis , an amino group, an amino group protected with a protecting group in nucleic acid synthesis , an alkoxy group having 1-6 carbon atoms, an alkylthio group having 1-6 carbon atoms, a cyanoalkoxy group having 1-7 carbon atoms or an amino group substituted by an alkyl group having 1-6 carbon atoms).

B represents a purin-9-yl group or a 2-oxo-1,2-dihydropyrimidin-1-yl group each of which is optionally substituted with 1 or more substituents selected from the following α-group].

(α-Group)

a hydroxy group,
a hydroxy group protected with a protecting group in nucleic acid synthesis,
an alkoxy group having 1-6 carbon atoms,
a mercapto group,
a mercapto group protected with a protecting group in nucleic acid synthesis,
an alkylthio group having 1-6 carbon atoms,
an amino group,
an amino group protected with a protecting group in nucleic acid synthesis,
an amino group substituted by an alkyl group having 1-6 carbon atoms,
an alkyl group having 1-6 carbon atoms, and
halogen atom.

2 A compound according to Claim 1, wherein R^1 represents a hydrogen atom, an aliphatic acyl group, an aromatic acyl group, a silyl group, a methyl group substituted by 1 to 3 aryl groups, or a methyl group substituted by 1 to 3 aryl groups wherein the aryl rings are substituted by a lower-alkyl group, lower-alkoxy group, halogen atom or a cyano group.

3. A compound according to Claim 1, wherein R^1 represents a hydrogen atom, a silyl group, a methyl group substituted by 1

to 3 aryl groups, or a methyl group substituted by 1 to 3 aryl groups wherein the aryl rings are substituted by a lower-alkyl group, lower-alkoxy group, halogen atom or cyano group.

4. A compound according to Claim 1, wherein R^1 represents a hydrogen atom, trimethylsilyl group, t-butyldimethylsilyl group, t-butyldiphenylsilyl group, benzyl group, triphenylmethyl group, 4-methoxybenzyl group, 4-methoxyphenyldiphenylmethyl group, a 4,4'-dimethoxytriphenylmethyl group, or 4,4',4''-trimethoxytriphenylmethyl group.

5. A compound according to Claim 1, wherein R^2 represents an azido group, an amino group, or a group represented by the formula -NH-R^3 (wherein R^3 represents an aliphatic acyl group, an aromatic acyl group, a methyl group substituted by 1 to 3 aryl groups, a methyl group substituted by 1 to 3 aryl groups wherein the aryl rings are substituted by lower-alkyl group, lower-alkoxy group, halogen atom, or cyano group, a silyl group, a phosphoramidite group, a phosphonyl group, a phosphoric acid group or a phosphoric acid group protected with a protecting group in nucleic acid synthesis).

6. A compound according to Claim 1, wherein R^2 represents an azido group, an amino group, or a group represented by the formula -NH-R^3 (wherein R^3 represents an acetyl group, trifluoroacetyl group, benzoyl group, benzyl group, p-methoxybenzyl group, tert-butyldiphenylsilyl group, a group represented by the formula $-P(OC_2H_4CN)(NCH(CH_3)_2)$, a group represented by the formula $-P(OCH_3)(NCH(CH_3)_2)$, a phosphonyl group, or a 2-chlorophenyl- or a 4-chlorophenylphosphonic acid group).

7. A compound according to Claim 1, wherein R^2 represents an azido group or an amino group.

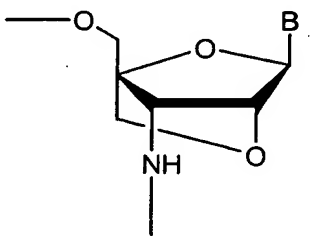
8. A compound according to Claim 1, wherein B represents 6-aminopurin-9-yl (*i.e.*, adeninyl), 6-amino-purin-9-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 2,6-diaminopurin-9-yl wherein one or both amino group(s) are protected with a protecting group in nucleic acid synthesis, 2-amino-6-chloropurin-9-yl, 2-amino-6-chloropurin-9-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 2-amino-6-fluoropurin-9-yl, 2-amino-6-fluoropurin-9-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 2-amino-6-bromopurin-9-yl, 2-amino-6-bromopurin-9-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 2-amino-6-hydroxypurin-9-yl (*i.e.*, guaninyl), 2-amino-6-hydroxypurin-9-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 6-amino-2-methoxypurin-9-yl, 6-amino-2-methoxypurin-9-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 6-amino-2-chloropurin-9-yl, 6-amino-2-chloropurin-9-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 6-amino-2-fluoropurin-9-yl, 6-amino-2-fluoropurin-9-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 2,6-dimethoxypurin-9-yl, 2,6-dichloropurin-9-yl, 6-mercaptopurin-9-yl, 6-mercaptopurin-9-yl wherein the mercapto group is protected with a protecting group in nucleic acid synthesis , 2-oxo-4-amino-1,2-dihydropyrimidin-1-yl (*i.e.*, cytosinyl), 2-oxo-4-amino-1,2-dihydropyrimidin-1-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 4-amino-2-oxo-5-fluoro-1,2-dihydropyrimidin-1-yl, 4-amino-2-oxo-5-fluoro-1,2-dihydropyrimidin-1-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 4-amino-2-oxo-5-chloro-1,2-dihydropyrimidin-1-yl, 4-amino-2-oxo-5-chloro-1,2-dihydropyrimidin-1-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 2-oxo-4-methoxy-1,2-dihydropyrimidin-1-yl, 2-oxo-4-mercapto-1,2-dihydropyrimidin-1-yl, 2-oxo-4-mercapto-1,2-

dihydropyrimidin-1-yl wherein the mercapto group is protected with a protecting group in nucleic acid synthesis , 2,4-dihydroxypyrimidin-1-yl (*i.e.*, uracilyl), 2,4-dihydroxy-5-methylpyrimidin-1-yl (*i.e.*, thyminyl), 4-amino-5-methyl-2-oxo-1,2-dihydropyrimidin-1-yl, or 4-amino-5-methyl-2-oxo-1,2-dihydropyrimidin-1-yl group wherein the amino group is protected with a protecting group in nucleic acid synthesis.

9. A compound according to Claim 1, wherein B represents 6-benzoylaminopurin-9-yl, adeninyl, 2-benzoylamino-6-hydroxypurin-9-yl, guaninyl, 2-oxo-4-benzoylamino-1,2-dihydropyrimidin-1-yl, cytosinyl, uracilyl or thyminyl.

10. A compound according to Claim 1, which is selected from the following:
3'-amino-3'deoxy-2'-O,4'-C-methylene-5-methyluridine,
3'-azido-3'deoxy-2'-O,4'-C-methylene-5-methyluridine,
3'-azido-5'-O-tert-butyldiphenylsilyl-3'-deoxy-2'-O,4'-C-methylene-5-methyluridine,
3'-azido-3'deoxy-5'-O-(4,4'-dimethoxytrityl)-2'-O,4'-C-methylene-5-methyluridine and
3'-amino-3'-deoxy-5'-O-(4,4'-dimethoxytrityl)-2'-O,4'-C-methylene-5-methyluridine.

11. An oligonucleotide analogue or a pharmaceutically acceptable salt thereof having 1 or more structural units represented by the following general formula (1a),



(1a)

provided that when the oligonucleotide has two or more structural units of formula (1a), each B is the same or different.

[wherein, B represents a purin-9-yl group or a 2-oxo-1,2-dihydropyrimidin-1-yl group which are optionally substituted with substitutents selected from α group below.].

(α group)

a hydroxy group,

a hydroxy group protected with a protecting group in nucleic acid synthesis ,

an alkoxy group having 1-6 carbon atoms,

a mercapto group,

a mercapto group protected with a protecting group in nucleic acid synthesis,

an alkylthio group having 1-6 carbon atoms,

an amino group,

an amino group protected with a protecting group in nucleic acid synthesis,

an amino group substituted by an alkyl group having 1-6 carbon atoms,

an alkyl group having 1-6 carbon atoms, and

a halogen atom.

12. An oligonucleotide analogue or a pharmaceutically acceptable salt thereof according to Claim 11, wherein B represents 6-aminopurin-9-yl (*i.e.*, adeninyl), 6-aminopurin-9-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis , 2,6-diaminopurin-9-yl, 2-amino-6-chloropurin-9-yl, 2-amino-6-chloropurin-9-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 2-amino-6-fluoropurin-9-yl, 2-amino-6-fluoropurin-9-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis , 2-amino-6-bromopurin-9-yl, 2-amino-6-bromopurin-9-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis , 2-amino-6-hydroxypurin-9-yl (*i.e.*, guaninyl), 2-amino-6-hydroxypurin-9-yl wherein the amino and hydroxyl groups are protected with a protecting group in nucleic acid synthesis , 6-amino-2-methoxypurin-9-yl, 6-amino-2-

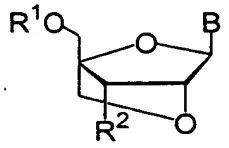
chloropurin-9-yl, 6-amino-2-fluoropurin-9-yl, 2,6-dimethoxypurin-9-yl, 2,6-dichloropurin-9-yl, 6-mercaptopurin-9-yl, 2-oxo-4-amino-1,2-dihydropyrimidin-1-yl (*i.e.*, cytosinyl), 2-oxo-4-amino-1,2-dihydropyrimidin-1-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 2-oxo-4-amino-5-fluoro-1,2-dihydropyrimidin-1-yl, 4-amino-2-oxo-5-fluoro-1,2-dihydropyrimidin-1-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis, 4-amino-2-oxo-5-chloro-1,2-dihydropyrimidin-1-yl, 2-oxo-4-methoxy-1,2-dihydropyrimidin-1-yl, 2-oxo-4-mercapto-1,2-dihydropyrimidin-1-yl, 2-oxo-4-hydroxy-1,2-dihydropyrimidin-1-yl (*i.e.*, uracinyl), 2-oxo-4-hydroxy-5-methyl-1,2-dihydropyrimidin-1-yl (*i.e.*, thyminyl), 4-amino-5-methyl-2-oxo-1,2-dihydropyrimidin-1-yl (i.e., 5-methylcytosinyl), or 4-amino-5-methyl-2-oxo-1,2-dihydropyrimidin-1-yl wherein the amino group is protected with a protecting group in nucleic acid synthesis.

13. An oligonucleotide analogue or a pharmaceutically acceptable salt thereof according to Claim 11, wherein B represents 6-benzoylaminopurin-9-yl, adeninyl, 2-isobutylamino-6-hydroxypurin-9-yl, guaninyl, 2-oxo-4-benzoylamino-1,2-dihydropyrimidin-1-yl, cytosinyl, 2-oxo-5-methyl-4-benzoylamino-1,2-dihydropyrimidin-1-yl, 5-methylcytosinyl, uracinyl or thyminyl.

Abstract

This invention provides novel bicyclonucleoside analogues which exhibit anti-AIDS activity and intermediates to produce oligonucleotide analogues which have anti-sense or anti-gene activity as well as in vivo stability.

The present invention is compounds of the following formula (1) or their pharmaceutically acceptable salts.



(1)

R^1 represents a hydrogen atom or protecting group for a hydroxy group,

R^2 represents an azido group or an optionally protected amino group or the like,

B represents a purin-9-yl or a 2-oxo-1,2-dihydropyrimidin-1-yl group which are optionally substituted with substituents selected from α group shown below.

(α group)

halogen atom, alkyl group having 1-6 carbon atoms, hydroxy group, mercapto group, amino group, and the like.